STUDENTS' REPORTS

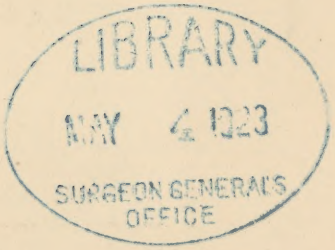
on Assigned Topics in

Histology

DEPARTMENT OF ANATOMY

COLLEGE OF PHYSICIANS AND SURGEONS

1923



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PREFACE

The present volume includes the third series of student papers prepared and presented by members of the first year class in connection with the course in Histology and Embryology. This feature was introduced several years ago as an experiment intended primarily to stimulate the student's interest in anatomical science by giving him some work for which he was entirely responsible. The enthusiastic response of the students and the high quality of the papers presented were very gratifying, and we feel strongly inclined to incorporate this work as a permanent feature of the course. Pedagogically, there can be no doubt as to its educational value. The student is familiarized with the methods of exploring the literature of a special field. He is trained in the proper selection and evaluation of material read, and in the organization of such material into a constructive and unified summary. The experience of the last three years has convincingly demonstrated that such work furnishes an excellent means for stimulating and sustaining the initiative and interest of the student.

The class desires to thank Professor Huntington for his kind permission to reprint a number of his previously published illustrations.

ADOLPH ELWYN.

Department of Anatomy
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THE NATURE AND FUNCTION OF THE PITUITARY BODY

IN man the pituitary body, or hypophysis cerebri, is located slightly anterior to the centre of the head. It is a gland, ovoid in general shape, and nearly as small as a pea. These things have been known for several centuries—but an idea of the nature and function of the pituitary body has been attained only in the past few decades. Vesalius, who lived some four hundred years ago, and whom Sir William Osler put down as "the real maker of modern anatomy," gave the name "pituitary" because he believed the gland secreted pituita, which is a term from the Latin and means nasal phlegm. A cold in the head was due to an increased function of this organ, supposedly. But Vesalius was incorrect in this view; and we now know the pituitary body to be essentially an endocrine or gland of internal secretion or ductless gland. Certain death follows its destruction; certain diseases and disfigurations follow its distortion. In 1886, Marie distinguished between acromegaly and gigantism—two abnormal conditions which we will include among others. Oliver and Schäfer, nine years later, in 1895, showed that an injection of an extract of the pituitary body causes a rise in blood pressure. The remarkable influence of this small structure upon the processes of development and metabolism is now realized after these discoveries. The pituitary is very old phylogenetically as well as embryologically.

As a preliminary to the nature and function, let us consider in some detail the anatomy of the gland in question—including the macroscopic or gross, and the microscopic or histological anatomy. Experiments will be cited. Then clinical cases of deranged pituitary will be taken up and therapeutic uses of injections of its extract.

Anatomy of the Normal Adult Human Pituitary Body

The long axis is directed transversely and measurers about a half an inch—while the measurement antero-posteriorly is approximately one-quarter of an inch. From above downward it is also about a quarter-inch. The total weight is normally about 0.5 gram. It is composed of two lobes—a large anterior lobe and a smaller posterior lobe. These are closely applied the one to the other, and Cushing observes that the posterior lobe fits into the anterior like a baseball into a catcher's mit. The infundibulum, or stalk of the hypophysis, which extends downward and somewhat anteriorly from that portion of the base of the brain designated the tuber cinereum, is attached to the posterior lobe. The midsagittal plane bisects the pituitary body and stalk into two more or less equal halves. Some difficulty is experienced in dissecting out the gland due to its softness and the adhesion of its capsule to adjacent structures. It is not easy to distinguish the anterior from the posterior lobe superficially, except that the anterior is the darker in color usually, due to its greater vascularity—the color approaching a brick red. A slight furrow some-

times marks off the two lobes, the one from the other. The blood supply for the anterior lobe is from small hypophyseal branches coming from each of the two internal carotid arteries as they pass immediately lateral to the hypophysis or pituitary body. Blood is carried to the posterior lobe via minute branches from the circle of Willis. Innervation is probably through twigs from the carotid plexus, which is sympathetic in nature, and surrounds the internal carotid artery.

Briefly, the organ under discussion is lodged in that depression in the superior surface of the body of the sphenoid bone known as the fossa hypophyseos. Bone lies in front of, below, and behind the gland, in general. This is referred to as the sella turcica (sometimes spelled tursica), which signifies an oriental or Turk's saddle. The back of the fossa is overhung by the dorsum sellae which anteriorly and on either side gives rise to a prominent tubercle—the posterior clinoid process. Similarly tubercles project backward from anteriorly, partly enclosing the fossa hypophyseos superiorly. The anterior wall of the fossa, from above downward is called the sulcus chiasmatis (for the lodgment of the optic chiasm) and the tuberculm sellae. Further anterior to the fossa are the superior parts of two air spaces—the sphenoidal sinuses—partitioned off from one another vertically, and which do not communicate posteriorly. These must be opened, in approaching the hypophysis when operations are performed upon it via the nasal route.

Lateral to the pituitary body are the cavernous venous sinuses—each having embedded in its lateral wall the internal carotid artery with its sympathetic nerve plexus, and the following cranial nerves: (1) The oculomotor (III); (2) the trochlear (IV); (3) the ophthalmic and maxillary divisions of the trigeminal (V); and (4) the abducent (VI). Sometimes hypophyseal tumors cause pressure upon these structures, giving rise to certain signs and symptoms. The floor of the hypophyseal fossa is on a level with a plane projected backward from the nasion (the middle of the naso-frontal suture) to the inion (the external occipital protuberance). *The distance from nasion to the pituitary body is from 2¼ to 2½ inches.* The roof of the fossa is a fibrous membrane fastened to the four posts of the sella turcica and having a small horizontal aperture for the passage of the infundibulum from the base of the brain to the posterior lobe.

Histologically, the anterior lobe consists of solid branched epithelial cords, of irregular caliber, and which frequently anastomose. These cords are separated from one another by wide lacunar capillaries derived from the several arterioles. The wide terminal vessels are arteriovenous connections having a sinusoidal structure. Particularly in the central portion of the lobe, the cords are covered with eosinophilic cells of two types—large granular and small oxyphiles having little cytoplasm. The former type seems to stain darkly with Wigert's elastic tissue stain. Both types of eosinophiles have round nuclei. The axial cells of the cords are neutrophilic and less granular. Basophile cells occur at the periphery of the anterior lobe.

The posterior lobe consists of a mass of neuroglia cells, the *pars nervosa*, and an epithelial investment, the narrow *pars intermedia*. The cells of the latter are basophile sometimes ciliated and tend to become cyst-like, the cysts containing "colloid" secretion. The pars nervosa contains neuroglia and ependymal cells, but no nerves and only a few nerve fibres. The ependyma lines the cavity which extends downward into the lobe from the infundibulum.

Tilney's division of the gland, compared with the terminology used above, follows:

A. Pars Buccalis or Glandularis:
   1. Pars distalis (anterior lobe)
   2. Pars juxta—neuralis (Intermediate portion)
      (a) Pars tuberalis
      (b) Pars infundibularis.

B. Pars Neuralis (Posterior Lobe—pars nervora above)
   (i) Eminentia saccularis
   (ii) Infundibulum
   (iii) Processus infundibuli.

Function

Although much work has been done on the function of the hypophysis, the results in many cases have been conflicting, mainly because the "active principles" have been never successfully isolated. Our knowledge is based on the results of extirpation, or of feeding dessicated gland and injection of glandular extracts. As the functions of the pars anterior on one hand, and the pars intermedia and pars nervosa on the other, appear to be quite different, they shall be considered separately.

Function of Anterior Lobe.

The pars anterior has been found to be concerned largely with the development of the skeleton and general growth of the body, especially the development of the sexual glands.

The removal of the anterior lobe of the dog causes coma and death, generally within three days. However, if some of the tissue is left intact the dogs do not die but develop the following symptoms:

1. Sudden cessation of growth.
2. Disturbances in ossification.
3. Exaggerated adiposity.
4. Genital hypoplasia.

Inversely Goetch found that by feeding anterior lobe to young rats there was a stimulation of growth, and a development of sexual glands. Increase in sexual activity has been noted as an effect of anterior lobe administration in man, and there is a hyperplasia of the anterior lobe in women during pregnancy.

Much of our knowledge of the function of the anterior lobe especially has been derived from the observation of cases having over secretion of the anterior lobe autacoid, hyperpituitarism; or having an insufficiency of secretion, hypopituitarism.

Over secretion of the anterior lobe early in life causes in the boy sexual precocity, and in the girl precocious masculinity, as made evident by hypertrichosis and deep voice. But the most apparent differences in functional activity are the disturbances in ossification. When the onset of hyper hypophysism occurs before the epiphyses have joined a remarkable growth of the skeleton follows and gigantism is the result.

If the disease commences later in life when the epiphyses have fused, gigantism does not occur, but there are changes in the bones of the head, face, hands, feet and thorax, acromegaly, which will be described later. Blair Bell believes that true gigantism cannot be considered pathological in the absence of acromegaly, for the giants produced are hypermetric, but no one

part is abnormally developed. It may, in fact, be due merely to adolescent hyperhypophysism just as one sees hyperthyroidism in girls at puberity, a condition which produces a temporary effect that subsequently subsides. Mason says that the difference between gigantism and acromegaly is explained perhaps by a different evolution of the cellular hyperplasia of the anterior lobe. When proliferation follows the normal, gigantism appears; when it is atypical acromegaly results. In other words, gigantism is due to hyperpituitarism, and acromegaly to dyspituitarism.

Concerning the results of underfunctioning of the anterior lobe, less is definitely known. To this hypopituitarism of the anterior lobe has been ascribed the dystrophy adiposogenitalis syndrome (to be described later), which is characterized by a tremendous increase in fat, and retardation of sexual development. However, Cushing believes this to be due to the under secretion of the posterior lobe. Blair Bell believes dystrophy adiposogenitalis to be caused by a general hypopituitarism for both lobes, for whole gland extracts are the best medicinal treatment for relief, and the excessive carbohydrate tolerance in these cases is due to under secretion of the posterior lobe.

It is not known whether or not hypopituitarism is a congenital lesion, for it is not until the child is growing up that signs of this lesion become recognizable.

Hypopituitarism before puberty gives rise to three conditions:

1. Infantilism (somatic and sexual) without adiposity.
2. Stunted growth with sexual infantilism and adiposity.
3. Overgrowth with some adiposity and genital inactivity.

The first case, Cushing believes is due to insufficiency of pars anterior alone, but 2 and 3 may be due to general hypopituitarism of the whole gland.

A chemical unit of unknown composition, was isolated by Robertson in 1916, known as tethelin, the use of which is said to be equivalent in all ways to the administration of the whole anterior lobe, and should be of clinical value. However there is no proof that this is the "active principle" of the anterior lobe. When tethelin is decomposed by mild hydrolysis, inosite is found among the products of the reaction. This is interesting from the fact that it is a constituent of rapidly growing parts of plants. Tethelin stimulates growth in young animals just as anterior lobe extract, and is believed to have a hastening action in the healing of wounds.

There has been no experimental evidence showing physiological activity in the pars tuberalis.

Function of Posterior Lobe.

The pars intermedia will be considered with the pars nervosa because there has been much evidence to show that the secretion of the former gathers in the latter. It has been theoretically advanced that the epithelium of the pars intermedia secretes a thin colloid which finds its way to the pars nervosa and is there stored as a hyaline material.

The function of the posterior lobe (including pars intermedia and pars nervosa) as observed by the administration of posterior lobe extract is as follows:

1. *Pressor action.* Oliver and Schäfer discovered that posterior lobe extract increases blood pressure with a small decrease in heart rate and strengthening of the systole. Its action is less intense but lasts longer than that caused by epinephrin. The secretion of the pars intermedia is thought

to have a depressor action for a depressor substance has been isolated in this part. Bell believes that this depressor substance is changed to pressor as it goes back to the posterior lobe. At any rate the resultant action of the secretion of the posterior lobe is pressor. The active principles of the posterior lobe are present at an early age; and pressor responses are made from foetal hypophyseal extracts.

2. *Stimulation of contraction in smooth muscle.* Certainly the most important therapeutic use of posterior lobe extract is its constrictor action on uterine masculature. It also causes an increase in milk flow of the mammary gland. This is not a true galactogogue effect, because the increased flow of milk stops as soon as the supply of milk present in the lumens of the glandular alveoli is forced out by contraction of the smooth muscle fibres about the alveoli, which is caused by the action of the posterior lobe extract. It also stimulates peristalsis. This was ascertained by Zondek in 1920, who by fitting a celluloid window in the abdomen of dogs observed the intestine directly.

3. *Carbohydrate metabolism.* The posterior lobe secretes an autacoid which lowers the tolerance for sugar, and the injection of this extract will lower sugar tolerance until glucosuria becomes established. Glycosuria often follows an operation in which the posterior lobe has been stimulated by manipulation. If the posterior lobe is removed, however, there will be an increased sugar tolerance to the extent that diabetes will not result even after the removal of the pancreas. It has been suggested that this increased tolerance for sugar is the cause of exaggerated adiposity in cases of hypopituitarism, which must involve the posterior lobe. Polyuria is generally associated in these cases with glycosuria and may be caused by the effect of posterior gland extract upon the convoluted tubules of the kidney themselves. In regard to carbohydrate tolerance Cushing says, "In our canine experiments it was noted that a transient spontaneous glycosuria was often the immediate sequel of certain operative manipulations involving the posterior lobe, but contrary to our expectations further study showed that instead of a persistent lowering of the assimilation limit for sugars the tolerance ultimately rose and remained far above normal. This was shown moreover, to be a consequence of posterior rather than of anterior lobe deficiency.

"It was subsequently observed that patients suffering from unmistakable hypophyseal deficiency associated with destructive pathological processes similarly showed a high, often an extraordinary tolerance for sugars—these usually being patients who had acquired or who were acquiring adiposity. Further, a majority of the individuals who exhibited evidences of overgrowth from hyperpituitarism (acromegaly and gigantism) were found to possess not a lowered but a high tolerance—an indication of the fact that at least so far as the posterior lobe was concerned they were passing from a state of increased glandular activity to one of lowered activity.

"The matter was further tested both in the laboratory and clinic, by atoning for the functional deficiency through the administration of glandular extracts. It was found as Borchard had previously observed, that the intravenous injection of posterior lobe extract in normal animals could produce glycosuria, and, what was more to the point, that the assimilation limit, which had been greatly raised in consequence of the removal or obstruction of the posterior lobe, could be again lowered to a normal, or even to a subnormal, level by injections or oral administration of extracts (those of the posterior

lobe proving far more efficient in this respect than those of the pars anterior, of thyroid or of adrenal)."

Other effects of posterior lobe administration have been noticed, not of such great importance, and about which less is known. In regard to further influence on kidneys, an intravenous injection of posterior lobe extract is said to be a diuretic; while an intramuscular or subcutaneous injection is said to be a depressor and an antidiuretic. An effect on respiration has been recorded, possibly due to a constriction of bronchial musclature. Also the secretions of the liver, pancreas, and salivary glands are said to be inhibited by posterior lobe extract.

The attempts at isolating in a pure indentifiable condition the active principles of the posterior lobe have been unsuccessful. It was formerly believed that histamin (beta imidazolylethylamin) was the plain muscle stimulating constituent of the pituitary gland, but it is questionable if this is present in the perfectly fresh gland.

In 1914 Führer was able to obtain a large series of crystalline compounds by precipitation of the protein free extracts of the material with phosphotungstic acid, and then treating this mixture to get sulfate salts. The mixture he named "hypophysin" and considered it to contain between four and eight active principles of the posterior lobe.

In addition there are many posterior lobe extracts on the market, commonly known as "infundibulin," although this name is quite misleading. Blair Bell suggests the use of infundibulin in shock for maintenance of blood pressure; also in collapse, sepsis, serum sickness, menopausal flushings, and asthenia. Its greatest therapeutic use is in the acceleration of labor, and it is also valuable in the prevention of post partum hemmorhage by contracting the uterus. It has been used in Cesarean section (a few drops applied directly to the uterus for a bloodless field). Infundibulin has been used in paresis of the bladder; and with more doubtful results to prevent paralytic distension of the stomach and intestine after abdominal operations. Injection of posterior lobe solution diminishes thirst, volume of urine, and increases the specific gravity of the urine in cases of diabetes insipidus. Local application is effective against capillary hemorrhage, next in value to epinephrin. Oral administration in all cases is practically ineffective, for posterior lobe extract, although not acted upon by pepsin, is destroyed by trypsin.

There is a synergy of the pituitary gland and the other endocrines. There is hypertrophy after thyriodectomy, after castration, or removal of the adrenals.

Experiments On Amphibia

Some valuable work has been done on the effect of extirpating the hypophysis of Anurans at a very early age, and observing the subsequent changes in development. Anurans were used because in these forms it is possible to ablate the epithelial anlage of the hypophysis at the time of its first appearance as an invagination of the oral ectoderm. In Allen's experiments the hypophyseal anlage was removed by making a transverse frontal cut exposing the ventral surface of the pituitary ingrowth. This was an extremely delicate operation for the Anuran larvae were but from 3.5 to 4 mm. in length, and the medullary folds were just closing. Rana pipens, the common frog, was used in these experiments. Very clean cut experimental results were obtained because of the great number of individuals used, the rapidity of development, and the ease of regulating controls and environmental conditions. The anlage

from the buccal ectoderm gives rise to the pars anterior, pars intermedia; and pars tuberalis. In all cases the pars nervosa was not removed, and the Anurans in which the hypophyseal anlage has been extirpated, but which still contain the pars posterior, shall be referred to for convenience as "pituitaryless" tadpoles.

I. *Removal of Anterior Lobe Anlage.*

Allen's results of extirpating the anterior lobe anlage were as follows:

1. Retardation of Growth. Size very small in comparison with controls. Limb buds extremely slow in development.

2. Failure to metamorphose.

3. "Silvering"; that is, the epithelium changed from its normal dark color to a creamy silver color.

4. Extremely high mortality. Of 30 individuals rendered pituitaryless, 4 remained alive in 33 days. In another set, of 100 individuals rendered pituitaryless, 7 remained alive in 32 days. It is probable that when the operation removed the entire anlage, death invariably resulted. Smith had similar results in independent experiments.

II. *Transplantation of Adult hypophysis to young Rana pipens larvae.*

The following experiments by Allen were based in their results on 384 operations. The tissue ingrafted was from adult frogs and contained all parts of the pituitary gland but the pars tuberalis. The ingrafted tissue was placed in a pocket under the skin below the right eye. When this tissue was transplanted to pituitaryless tadpoles they became larger than the controls, and there was acceleration in the development of the hind leg buds.

The intermediate lobe alone was implanted in pituitaryless tadpoles, with the result that their abnormal silvery color was changed again back to the normal dark color.

The intermediate and posterior lobe was implanted in pituitaryless tadpoles, with the result that the silvery color changed back to dark, and there was a general contraction of the body walls which gradually disappeared.

The posterior lobe alone was implanted in pituitaryless tadpoles with the result that they remained silvery in color, and there was a general contraction of the body walls which gradually disappeared.

These results may be summarized as follows:

1. The anterior lobe of the hypophysis stimulates growth and metamorphosis.

2. The intermediate lobe is very largely concerned in regulating such color changes as are controlled by the hypophysis.

3. The posterior lobe causes marked contraction of the body walls.

The effects of 1 and 3 are most interesting because of their great similarity to what we know about the function of the anterior and of the posterior lobe of the human pituitary gland.

The effect of 2, as far as we know has no exact counterpart in the function of the human pituitary gland, thought it may be said that increase in pigmentation is often present in cases of acromegaly.

In regard to silvering in the tadpole, Swingle says, "It remained for Atwell ('19) to show that it is the lack of the pars intermedia that is responsible for the albino appearance of hypophysectomized larvae. He extirpated the hypophysis of the frog embryo and, following the silvering of

the tadpoles places them in a dilute extract of pars intermedia of beef pituitary. The animals soon underwent a striking color change from silvery to dark, in the latter condition closely resembling normal tadpoles." The silvering is brought about, according to Swingle, by changes in the pigment bodies of the epithelium. Extirpation of the anterior lobe causes the more superficial pigment bodies, which contain guanine and xanthine, to spread out and ramify in an arborescent manner, thus giving the creamy silver effect so marked in hypophysectomized tadpoles. In addition extirpation of the anterior lobe causes the deeper pigment bodies, the melanophores which are black, to shrivel up and become more condensed. Inversly, implantation of the pars intermedia in hypophysectomized tadpoles, causes contraction of the zantholeucophores, which contain the superficial, light colored pigment.

Diseases

The results of diseases of the ductless glands in general may be shown in:

(1) Disturbances of the gland in question.

(2) Secondary disturbances in other glands (because all the endocrines are bound together, as it were), causing a polyglangular syndrome (syndrome = sum total of symptoms).

(3) Involvement of the vegetative nervous system, and therefore the influence on many organs.

Pituitary syndromes are characterized by:

A. Dystrophic Conditions
   (1) Acromegalia (acromegaly)
   (2) Gigantism
   (3) Pituitary Infantilism.

B. General Disorders of Nutrition
   (1) Dystrophy Adiposogenitalis
   (2) Pituitary Diabetes.

A-1 *Acromegalia,* also spelled acromegaly, according to Marie, is a "noncongenital hypertrophy of the limbs and head." *The diagnosis may be made on sight.*

Symptoms

Face—Lengthened, having the appearance of an elongated oval.
Forehead: Low. *Supraorbital ridges prominent.*
Eyes: Small.
Nose: Enormous, the increase in size being in all directions.
Cheek bones prominent. Cheeks sunken.
Ears: Abnormally long.
Lips: Especially the lower, are thick and fall down.
Tongue: Hypertrophied. Sometimes interferes with swallowing.
Mandible: Excessive development. Chin stuck forward.

Head—Cranium—Little changed. Anterior-posterior diameter slightly increased. Voluminous external occipital protuberance (inion). Increased height and depth of frontal and maxillary sinuses.

Upper Extremities—Arms and forearms normal. Wrists slightly hypertrophied.

Hands—characteristic deformities. Large, thick and *spade-like.* Both the bones and the soft parts are hypertrophied. Apparently padded. Fingers short—sausage shaped. Square at ends and as large at ends

as at bases. Nails flattened. Short. Striated longitudinally and hardly cover dorsal surface of ungual phalynx. Lateral bony growth ⅔ distal on first phalanges of the fingers.

Lower Extremities—See upper extremities. Similar. Thigh and leg normal, *Enlargement from heel to toe.* Big toe larger in proportion.

Thorax—Malformation less constant. (Cervico—dorsal kyphosis, sometimes).

Skin—Sometimes altered—but, according to Osler, never harsh and dry looking as in myxedema (hypothyroidism). Often pigmented at level of the eyelids.

Hair—thick.

Articulations—Prominent and often seat of spasms.

Genital Disturbances—In the male: frigidity and often atrophy of the testicles.

In the female: menses decrease or stop.

Viscera—Splanchnomegalia often. Heart may enlarge without apparent cause. Liver and spleen also may hypertrophy.

Acromegaly is rare. It occurs slightly more frequently in men than in women. It begins at the age of 25 or 30, very insidiously. Twenty per cent of acromegalics are over six feet when the symptoms begin and fully forty per cent of the giants are acromegalics (Sternberg). Sometimes somnelence is the first symptom—and headaches are common throughout the course of the disease. Due to factors mentioned in the epitome of symptoms given above, the patients are forced to procure larger and larger hats, gloves, and shoes. Friends notice the facial deformities. The disease may last 20 to 30 years; in some cases, with brain tumor, death follows in three to four years. The etiology or cause is usually associated with a tumor of the pituitary body —nearly always an adenoma, adenosarcoma or a sarcoma. The pituitary increases in weight greatly. Trauma (injury), the infections and emotional shock have preceded the onset of the disease. The enlarged hypophysis usually extends *downward and forward* as the sella turcica enlarges in like manner as shown by X-Ray plates or upon postmortem examination. In summary, *acromegalia* presents certain changes in appearance notably of the *face, fingers and feet.*

A-2 Gigantism is due to hyperpituitarism, or over-function of the hypophysis. Uusually the person suffering from gigantism is over 6½ feet tall. There is a disproportion between the various segments of the body. It begins before the epiphyses have fused to the diaphyses, and is therefore different from acromegaly in this respect. The lower extremeties may elongate—the person appearing as if on stilts (macrosketic type) or the arms may become longer (bradysketic type). Temporary strength may be observed at first, but sooner or later the individual suffering from gigantism becomes weak, bodily and mentally—and asthenic. They walk with difficulty, and they nearly always are impotent and sterile.

Three varieties of forms are presented clinically:

1. Gigantism with acromegalia. Resemble anthropoid apes. Fingers not sausage-shaped, for the development is in length more than in width.

2. Infantile gigantism. Elongation of inferior extremities—with genu valgum. Genital organs infantile. Hairs do not appear as normally. Voice frail and childish. Feminine form of breasts (slight); abdomen (rounded);

pelvis (broad). Cartilages persist in the adult and the epiphyses remain unfused with the diaphyses. Gradually changed to acromegalia-gigantism.

3. Gigantism. Sometimes occurs. The persons have a large skeleton, but enjoy good health. They are normal giants whereas the two varieties (1 and 2 above) were pathological or abnormal giants. However, the intelligence is often mediocre; and sometimes impotence is a concomitant.

Gigantism, of whatever form, about always occurs in the male; at puberty or before puberty. Death usually occurs in youth, due to pulmonary tuberculosis after emaciation and cachexia. Uusaully a tumor (as in acromegaly) is found at autopsy, associated with an increase in the dimensions of the sella turcica.

A-3 Pituitary Infantalism is so uncommon that we will omit a discussion of this type of hypopituitarism or anterior lobe *insufficiency*. Suffice it to state that it is a general retardation of development, especially as regards genital growth.

B-1 Dystrophy Adiposo-genitalis, the first of the pituitary syndromes, listed under "General Disorder of Nutrition," was observed by Babinski in 1900. It occurs in infancy, adult life or even at menopause. Two symptoms stand out pre-eminently:

(1) Marked general obesity or "fattiness," and
(2) Delayed development or atrophy of the genitalia.

Symptoms—Fat invades the whole body.

Face large and rounded. Often cyanotic. Marked *fatty* hypertrophy of the breasts in women. The mammary glands atrophy, however.

Relaxation of abdominal wall.

Hips and buttocks sometimes enormous.

Limbs resemble columns.

Skin waxy white; hard, cold edematous and incompressible by the fingers. In this respect like myxedema (hypothyroidism).

In male children the testicles stay infantile. Secondary sex characters are absent. In women the menses become irregular and later a condition of amenorrhea (lack of menstruation) ensues. Frigidity and impotence take place in men.

The etiology of dystrophy adiposogenitalis is complex. The causes are many. There is evidence of thyroid and ovarian (or testicular) deficiency. Cushing believes the disease to be due to a lesion of the *posterior* lobe of the pituitary body.

B-2 Pituitary Diabetes. Marie, in 1886, stated that one-half the cases of acromegalia showed pituitary glycosuira (sugar in the urine), which may be continuous or intermittent. The exact portion of the pituitary body which is responsible for this is as yet unknown.

Pituitary polyuria has been noted. Canms and Roussy believe, after experiments on animals, that the water regulating centre is in the region of the infundibulum or tuber cinereum.

The Clinical Aspects of Pituitary Diseases

Concerning the disorders of this secretive gland of the head, the clinic has manifested that the five groups listed on page 25 of Dr. Cushing's book are the most instructive. In very general terms hypophyseal implication may be listed under two heads: (1) "Those in which the gland was seemingly

the primary seat of disease"; (2) "Those in which it was secondarily involved, usually by the direct compression of an adjoining part, or the more remote effects of a distant, cerebral lesion."

Cases may again be classified in general as Dyspituitarism; under which the implications may be graded according to the amount of secretion in relation to the normal; if over-secretion is shown the term hyperpituitarism is used; if the gland is under secreting the condition is called hypopituitarism.

Quite evidently disease of the pituitary tends to produce a change in the metabolistic activity of the organism as a whole; this fact is important, not only in a clinical way as regards the health and happiness of the patient, but also from the point of view of research in the field of the factors controlling the development of the organism. Turning now to the examination of two cases, selected from a book written by Dr. Harvey Cushing, entitled "The Pituitary Body and Its Disorders," a definite order will be followed in recording the data, namely: 1. Record of Case; 2. History; 3. Diagnosis; 4. Prognosis; 5. Discussion.

Case 2 (Surgical No. 25971) see page 36:

1. Record of Case—"Typical recent hyperpituitarism of adult life (acromegaly) with an actively-growing chromophobe struma, producing both pronounced neighborhood and general pressure symptoms. Operation. Death. Autopsy."

Symptoms of hyperpituitarism follow: Overgrowth, resulting in gigantism when process anterdates ossification of the epiphyses—typus Launois; resulting in acromegaly when it is of later occurrence—typus Marie.

2. History—1910. Seamstress, 25 years old was admitted; complaint, Amenorrhoea. Was one of a healthy family of twelve. Had been well except for single sickness in Russia seven years ago; in hospital then with "headache, fever and blindness." She regained her vision. Her menses began at 15—regular until her marriage, three years ago (1907). She dates existing malady 1907. Pregnant: temporarily blind in right eye. "Eleven months after her marriage the menses reappeared for four months, but since then amenorrhoea has been complete." Two years ago an enlargement of nose, hands and feet was first noticed. Headaches from onset, but now severe.

3. Diagnosis—See history. Headaches occur in paroxysmal attacks several times a day, accompanied by burning flushes and occasionally by nausea, projectile vomiting, temporary amaurosis and relaxation of the sphincters. No loss of consciousness. Periods of diplopia and vision rapidly failing. Sweating profuse for two months, particularly at night, with itching and burning of the extremities. Drowsy. Yawns. Sleeps most of the time. Has had attacks of palpitation and polyuria. Habitually constipated.

Physical exam.: Short (5 ft. 5 in.) overnourished woman, wt. 143 lbs. Expression is dull, stolid. She had normal heart and peripheral vessels; no arteriosclerotic changes. Skull was not particularly modified. Adiposity not a striking feature. Temperature slightly above nomal (99-99.6).

Sugar assimilation not high.

Thyroid is palpable, undersized uterus, only one small ovary is palpable.

Marked pigmentation of the skin.

4. Progonosis—Operation: "Hypophyseal decompression by the sublabial route with submucous resection of vomer. Terminal pressure phenomena of unsuspected intracranical extension of struma. Medullary failure on the following day."

5. Discussion—A postmortem examination showed that the patient had a tumor occupying the third ventricle of the brain; evidently produced in the hypophysis. Also a somewhat enlarged thyroid with a colloid goitre; ovaries are large, cystic and degenerated. Pancreatic islets numerous, large and the gland shows a fatty infiltration. Considerable persistent thymic tissue. Adrenals large. Fatty change in the liver. Small uterus.

"Her luxuriant hair, moist skin, elevated temperature, normal sugar tolerance and the insignificant thermic reaction to an injection of anterior lobe extract all pointed to a state of hypophyseal hyper-activity."

Case 30. (Surgical No. 26068). See page 154.

1. Record of Case—"Typical chronic advancing hyperpituitarism (acromegaly with the suggestion of gigantism) of fifteen years' duration. Absence of objective neighborhood symptoms. Subtemporal decompression. Glandular therapy."

2. History—June 8, 1911, admitted for study. Forty-two years old, a collegiate instructor. Younger sister has been suffering for some years with headaches, has a changed contour of face and undershot lower jaw. Patient himself periodic headaches since early youth. Attacks of hay-fever. Mucous membrances particularly sensitive to certain odors. "As a young man he was delicate-featured and light complexioned, weight 140 lbs. in 1892, when he graduated with high classical honors and started his career as a teacher. He has since grown dark, heavy and taller, weighing now over 200 lbs." Married in 1898; no offspring.

3. Diagnosis—Onset was insidious. His surgeon noticed it in 1896 when the patient was operated on for enlarged cervical glands. In 1900 intradranial discomforts pronounced. Since then he has taken pituitary extract, except between the years of 1904 and 1908, when he took thyroid extract. Headaches. No nausea nor vomiting. Complains of logginess. Low blood pressure. Progressive decline in libido sexualis for some years. Perspires freely. Overgrowth of bones as well as in their coverings. Head large. Nose shows the characteristic narrow bridge and wide nostrils. Lips are thick. Hands are spadelike. Feet large. Skin not pigmented but moist. Gained 60 lbs. since onset of malady. Temp. 97-98.

4. Prognosis—Operation. A right subtemporal decompression was performed. Headaches still persist.

5. Discussion—"At present the active process has subsided and the condition is verging toward hypopituitarism—at least on one side of the posterior lobe insufficiency, judging from the subnormal temperature, the low blood pressure, increase in sugar tolerance and the tendency toward adiposity. . . . The static headaches, invariably relieved by recumbency, are possibly due to the emptying of the fluid contents (cerebrospinal or venous) of the cranial chamber when the patient assumes an upright position."

Miscellaneous

Pituitary Syndromes in Infections are sometimes alleviated by pituitary organo-therapy (extracts from the hypophysis of the ox is usually used) by producing:

1. Increase of arterial pressure.
2. Increase of diuresis.
3. Decrease of tachycardia (= *rapid* heart rate).
4. Reappearance of sleep and appetite. Epinephrin or adrenalin (from the suprarenal bodies) is also often associated here.

Treatment of Pituitary Syndromes:

1. Organo Therapy.
   Posterior lobe extract. Average dosage, 0.10-0.40 centigrams, daily.
2. Radio-theraphy.
   Some success has followed this method (Beclere and Jaugeas).
3. Surgical Treatment.
   Nasal route is the best method of approach, but the fatality is still high. It may be indicated especially in acromegalia.

From this abridged discourse on the nature and function of the pituitary body one may rightly infer that a vast field of research on this particular endocrine gland still remains. Already, however, we are far ahead of the "cold-in-the-head" theory. We know something of its physiological, pharmacological and therapeutical actions; and of its diseased conditions. That a structure so small produces such relatively enormous influences upon the body merely emphasizes the ancient remark of David in Psalm 139, "I am fearfully and wonderfully made."

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MENDELIAN INHERITANCE IN MAN

IT has been customary to employ all scientific deductions for the explanation of human phenomena. This, of course, is only natural for what we are ultimately interested in is how this mechanism works. In general, the laws of physics and chemistry explain to us the purely physical and chemical reactions which go on in the body. From physics we understand the mechanics of this machine, and its energy transformations. So we speak of the skeletal system as being a system of finely adjusted levers. From chemistry we understand how the chemicals we take in are transformed into others which we can use. In short, all branches of engineering, mechanical, chemical, and possibly electrical, are comprised in the human body. The laws of biology and psycology, the former derived from the study of animals and plants, the latter from the lower animals and man, have been profitably brought into use. From the study of these sciences have developed others which deal exclusively with human material, deriving their data from human experience past and present, individually and collectively, and dealing with the two forces, heredity and environment.

We shall atttempt this morning to present a very brief sketch of the work already done on the explanation of the phenomenon of heredity in man by one of these principles.

In 1858, Darwin published his "Origin of the Species" and included an outline for heredity together with variation to establish the doctrine of evolution. He devised an ingenious theory which he called pangenesis, postulating that the different organs of the body gave off into the blood or other body fluids minute living particles which he called gemmules, and which he supposed to be capable of growth and multiplication. The germ cells were supposed to have a special affinity for these gemmules, their function being to act as storehouses for these bodies. During development the gemmules were sorted out, each kind determining the development of a part of the embryo into the kind of organ from which it was derived. He had accepted the doctrine of the transmission of acquired characters which Erasmus Darwin, and later Lamarck, had postulated as the chief cause of organic evolution. Granting Darwin's doctrine of Pangenesis, the explanation of the transmission of acquired characters followed very naturally but the difficulty lay in the artificial and improbable nature of its fundamental assumptions.

De Vries modified this doctrine and paved the way for Weismannism by eliminating the hypothesis of the centripetal flow of pangens and assuming that they were not given off by the cells of the body and stored up in the germ cells but the germ cells were held to receive their store of pangens from antecedent germ cells. Weismann, however, was the first to vigorously oppose Lamarckism. In 1892 appeared his "Doctrine of the Germ Plasm" in which he taught that the germ plasm is a substance separate from the soma plasm which forms the organs of the body, that it is in no way the product of the body, although it is carried and nourished by the body. Germ plasm is handed on relatively unchanged from one generation to the next, part of it being

transformed into soma plasm which differentiates in various way during embryonic development, but another part of it remaining undifferentiated in the germ cells to form the starting point of the next generation. It is the continuity of the germ plasm that affords the basis of heredity.

The discovery which has meant most in the study of heredity is unquestionably Mendel's law. The product of years of research in the garden of the monastery of Brün, Austria, the principles enumerated by Mendel, owing to the fact that they were published in a little known journal, "The Proceedings of the Natural History Society" at Brün and in the excitement of the post-Darwinian controversy, failed to attract the attention of the scientific world until they were made known independently by three investigators, Tschermack, Correns and De Vries, in the year 1900.

Mendel's law includes two main principles, the principles of dominance and segregation. Characters were found to be present in opposing pairs, as dwarfness and tallness in peas. When pure dwarf was crossed with a pure tall, the offspring were all tall, in other words, tallness is the dominant and dwarfness a recessive character.

	DD	RR		
F1	Dr	DR		
F2	DD	DR	DR	RR
		3		1

When the tall peas in F1 were crossed, the F2 generation gave tall and short peas similar to the grandparents in the proportion of three tall to one short. When these were analyzed it was found that one-half of the crop were similar to the parents or heterogeneous, containing characters for both tallness and shortness in the germ plasm; one-fourth of the crop were similar to the tall grandparent and bred only tall peas; one-fourth similar to the short grandparent and bred only short peas. In the F2 generation it is seen that the separate characters present in the germ plasm cells of the parent must have been separated and reunited. This is Mendel's law of segregation.

But it is not often an easy task to show this, especially in man, where complete dominance is not a general phenomenon. "Contrasted characters," says S. J. Holmes, "frequently blend in the first generation and many gradations occur between complete dominance and a strictly intermediate condition, but this in no wise alters the fact of segregation, although it may render segregation more difficult to establish."

Parallel with and entirely independent of the function of Mendel's laws the study of cytology and especially of the necleus and its contents give us the mechanism by which inheritance is maintained. We have as proof that heredity is determined by the nucleus and the chromatin material in it: the essential value of the nucleus in metabolism; the equivalence of the germ cell nuclei; the process of mitosis, the enucleate experiments and experiments with polyspermic eggs. In spite of this, Godlewski's classic experiment by which an offspring with maternal characteristics was obtained from an egg deprived of its nucleus and fertilized seems to point to the contrary. Recent investigators, however, have attempted to explain this by saying that the mitachondria in the cytoplasm have a function similar to the chromatin in the nucleus as bearers of heredity.

We are handicapped at the outset in man by having no experimental evidence, by the many difficulties of getting correct statistics, and by the frequent pitfalls offered to the unwary in their interpretation. The genealogies on which we must depend, the imperfect records of births and deaths, erroneous diagnosis, and sometimes, the uncertainty of the paternity of a given child, which is frequent among mental and moral defectives, hinder correct interpretation. Still other difficulties exist. For example, the very limited number of children of any given couple, the possibility of escaping the environmental stimulus which would call forth a trait, and then the peculiar character of certain traits, peculiar in that they appear only late in life. Furthermore, the study of inheritance is made extrmely difficult because many of the traits are due to multiple determiners. Morgan says "Often the best we can do in the case of man is to try to find the simplest Mendelian formula to which the evidence will fit. If one factor difference will not suffice, then two must be tried: if two will not do, then three must be tried, etc." However, numerous human traits are already known which give strong evidence of being transmitted in accordance with this law. A few of these will be discussed in detail and the rest tabulated.

Among the clearest cases of Medelian inheritance in man is the inheritance of certain malformations of the hands and feet. Among the first of these families to be investigated was one in Pennsylvania by Dr. Farabee in 1903. Though reports and histories had been recorded of such conditions since 1857, Farabee's report, we may say, marks the first investigation on the subject from the Mendelian standpoint.

The condition he described is known in the current literature as brachyohalangy or brachydactyly. This is a condition in which the middle row of phalanges, exclusive of the thumb, and big toe, is abortive. Dr. Farabee was under the impression that this row was entirely absent but Dr. Drinkwater, in his later analyses by means of radiographs, proved that it was present. In some cases ankylosis had occurred with the distal phalanx, so that it seemed to be missing. This, naturally, would result in an abnormal shortening of the fingers and toes to about one-half normal length. There is a corresponding shortening in the length of the thumbs and big toes, the relations remaining the same as in the normal hand. The general appearance of the hand is short, broad, thick and pulpy. This, in general, is the character of the malformation which is inherited, but there are many minor variations in the somatic appearance of this character.

These variations occur in the number and comparative size of the abortive middle phalanges, in the absence or presence of distal phalanges, in the character of the malformation of the thumb and big toe, in the absence or presence of finger nails and in the corresponding shortening of the metacarpals and metatarsals of the long bones of the upper and lower extremities evident in the span of the arms and in the shortening of the stature of the individual. The latter variation does not occur in members of the same family but in different families studied.

It is noticed that the malformation is always bilaterally symmetrical and when the feet were also examined, they showed corresponding abnormalities. The carpel and tarsal bones are not affected in any case.

In England and Scotland Dr. Drinkwater, in his extensive studies, has come across the same conditions. It was first brought to his attention in 1908.

A very interesting connection was established between his "second brachydactylous family" and Earabee's family in America. One of the affected individuals of his second generation migrated to America and became the father of the line of Farabee's family. Dr. Drinkwater also came across a condition that is not so marked where the shortening has not gone so far. This condition he called minor brachydactyly. Here there is no ankylosis and the relations can be seen from the diagrams.

This condition is transmitted by both sexes and is not confined to the white race. A similar malformation has been reported by H. M. Smith in a negro family and traced through four generations. The only accompanying character in some cases is the abnormal shortness of stature.

Though the discussion of the origin and cause of the malformation is not in direct line with the subject of this paper, we may say that Dr. Drinkwater believes it to have occurred as a "sport." The cause of this is probably a premature ossification, for where ossification is complete in the normal individual at the age of twenty-one, in some of the abnormal cases, the ossification is complete as young as twelve. The absence of epiphyses or the early union at the epiphyses or ankylosis, and a slight shortness in the shaft of the bone are other causes.

From the study of the inheritance of this malformation in several families through several generations, it seems to be transmitted as a Mendelian dominant. There is perfect segregation of the character, i.e., it is either inherited in full or not at all and is transmitted only by the affected individuals, for in no case has the character cropped up in the family of a normal individual whose parent was affected. This was well brought out in Farabee's family where in the third generation two normal cousins married and had only normal children, showing that the union of two recessives does not give a dominant.

If the character is a Medelian dominant, then 50 per cent of the offspring should be normal and 50 per cent abnormal, provided the abnormals marry normal individuals.

$$\frac{DR \times RR}{DR + 2RR}$$

Investigator	No. of Offspring	Normal	Abnormal	Percent
Dr. Farabbee	69	33	36	52.17
Dr. Drinkwater ..... 1st Bracydactylous	75	36	39	52
" " ..... I Minor Brachydactylous..	47	26	21	44.6
" " ..... II Minor Brachydactylous..	19	10	9	47.37
" " ..... II Brachydactylous	98	48	50	51.02
" " .....III Brachydactylous	42	17	25	59.52

Average Percent.....51.11

Another hand and foot deformity which by some is claimed to be a case of Mendelian inheritance is the case of split hand and split foot. This is usually a symmetrical clefting of the foot with a complete syndactyly or union of the remaining two groups of toes and an irregular though often symmetrical deformity of the hands. As in the preceding malformation there are many

minor somatic variations, but the general type of deformity is the same throughout, the variations being one of degree and not of kind.

The following are counts of split foot families by different investigators.

Investigator	No. of Offspring	Normal	Abnormal	Percent.
Parker & Robinson	32	16	16	50%
Lewis & Embleton	76	44	32	42.1%
Fotheby	26	16	10	38.5%
Mayer	18	12	6	33⅓%

Of the normal characteristics of man which mendelize, eye-color is perhaps the best established. The color of the eye acts as a unit factor, double in the zygote and separted in the germ cell into its original components. According to the kinds of germ cells which may then combine we find eye-color of either a homozyggus or heterozygous character. If brown meets brown an individual is formed with a double factor for brown. Again, if brown meets blue, we get an individual with brown eyes who has in his germ plasm a factor for blue.

As can be surmised from the above statements, evidence points to the fact that the brown color is dominant to gray, blue or albino. This is easily understood when we recall that the brown color of the eye is formed by melanic pigment in the front of the iris. Blue-eyed individuals lack this coat. They have a dark purple coat, on the inner surface of the iris. In the case of the albinos, the coat behind the iris which gives a blue color when light is transmitted through it, is also lacking. The pink eye-color is due to the blood capillaries present, which are not obscured by any pigment and which are seen through the semi-transparent fibrous tissue as pink.

Sometimes melanic pigment is scattered in granules in the front of the iris. The few granules upon the background of pigment which shows as blue gives gray eye-color. We also find, occasionally, that the iris contains a slight amount of yellow pigment which is presumably a fat pigment or lipochrome. This, with a blue background, gives green eye-color. We can sum all the evidence up by giving the nature of the mating.

Duplex	Duplex	All Duplex
Duplex	Simplex	50% Duplex, 50% Simplex
Simplex	Simplex	25% Duplex, 25% Nulliplex, 50% Simplex
Nulliplex	Nulliplex	All Nulliplex
Duplex	Nulliplex	All Simplex
Simplex	Nulliplex	50% Simplex, 50% Nulliplex

Duplex=DD Brown
Simplex=DR Brown
Nulliplex=RR Blue

The lack of the brown coat in the case of blue eyes is interesting in the light of history. In Northwestern Europe blue, gray and yellow-blue eyes are predominant. By noticing the distribution of eye-color the migrations of people can be traced. In the Spey valley of Scotland, we are told, the density of pure blue eyes is high—probably due to the Norse invasion at that point. Pigmentation of eyes and skin is also presumed to better fit a child for life in the tropics or in the bright sunlight.

An inheritance, similar to that of eye-color, is demonstrated in the case of skin pigmentation. Four kinds of pigment, black, yellow, red, and white are involved. In a slightly tanned white person the distribution of the pigment as shown when determined by a color top is

B	Y	R	W
8	9	50	33

In a very dark negro it is

75	3	20	2

The dark pigment is a melanic pigment found in fine granules in the deeper layers of the stratum mucosum of the skin. The pigment granules are discontinuous and the grades of color may be due to more or fewer elements of the so-called mosaic. The difference in negro, mulatto and brunet may be the difference in the point at which the melanogenetic process is stopped. The yellow and red pigment is considered as a lipochrome. The white color is due to the opacity of the skin itself.

In the case of the typical Causasians, it has been found that when both parents have blond complexion or fair skin all of their children will have fair skin. A brunet who has the double factor for dark pigmentation when mated with a blond, will have only intermediate and brunet offspring. Thus blond is seen to be the recessive character. The brunet coloring, if present dominates and there seems to be no blending of the colors as is the case in the F1 generation of the red and white mirabilis flower blending to give a pink color.

If this is so, then the problem of explaining the significance of intermediate color in the case of the white, arises. Sometimes this color has been found to act as a simplex, or heterozygous condition. In skin color there is more than one color factor involved. Therefore, in most of the literature we find that intermediate is termed epistatic to blond and hypostatic to brunet. When the determiner for intermediate is present in the germ cell and is linked with the factor for blondness, it either acts with blondness as part of the unit character and therefore gives no sign in a resulting soma of its presence, or again it may react as a separate unit and as a consequence obscure the factor for blond. In a similar manner intermediate is linked with brunet in the germ cell. In this case, brunet is the darker pigmentation and will obscure the intermediate unless the intermediate works as a separate unit.

The confusion concerning intermediateness arises because we do not know exactly how or why differences in amount of pigmentation occur. Pigmentation stops at a certain point which may be determined by an hereditary unit which halts melogenesis at that point. We do not know what this hereditary unit is or how it works. It has been thought that some such retardation or acceleration functions in giving to negro and white a different pigmentation. However, there is certainly a wide difference between the two, not a mere shading from one to the other, as in the case of negro-white crosses. What the difference is, is not known.

A very few years ago it was thought that the mulatto illustrated a case of so-called "blended" inheritance and once formed a constituted a distinct type. We find that, on the contrary, the original grades of heavy and slight melanogenesis segregate in the germ cell according to the Medelian formula. This segregation is often not entire because of the multiplicity of units for skin pigmentation. Thus the original color characteristics are restored in

almost their original purity in the gamete. To sum up the evidence briefly, when one parent is white and the other is as dark as a full-blooded negro, the offspring vary in color from black to as light as most Caucasian skin and follow the Mendelian principle. Thus in rare instances from the hybrid in a third or later generation a typical Caucasian, physical! and mentally, may result.

Inheritance of albinism also follows the same laws. Albinism has been found to be a recessive character. It occurs as a result of consanguineous matings, at which time two recessive traits come together. It is the product of an albinism mating and also occurs when the two strains have a marked absence of dark pigmentation.

Pathological conditions offer a much greater field for the indication of how Mendelian inheritance operates in man. Some conditions, as haemophilia, which is a proneness to hemorrhage and a difficulty in blood clotting, are sex-linked. These presumably have a determining factor linked in the X or sex determining chromosome. We know that a haemophilic person mated with a normal person has sons of whom half the number are haemophilic. None of the daughters have this condition, but one-half carry the defect in their germ plasm, which they can transmit to their sons. Normal sons do not transmit this characteristic. Predisposition to various diseases and the occurrence of various disease during the early or late years of an individual are also inherited in Mendelian fashion. These are not sex-linked. The literature on the subject is vast, but space does not permit us to describe them here. Some will be found in our table. Others can be studied by referring to the bibliography appended.

Perhaps what is more interesting to us as students of the human race is the inheritance of mental characteristics. Though there is a great deal of controversy in this field, a few general conclusions may be drawn from the large amount of data available. It must be remembered that the attempts to make these characters mendelize is of comparatively recent date. It has long been known that mental deficiencies are transmitted from parent to child, but no precise rules had been found. There is no doubt that all mental deficiencies are not due to environment alone. In the case of feeblemindedness, it has been shown through thorough psychological investigation of the individual and his family, that feeblemindedness is inherited. Since they tend to marry their own kind, the results of such unions to society can well be imagined. The most fruitful study along this line has been done by Dr. Goddard on the Kallikak family. One branch of the family has been prominent in all the intellectual and social walks of life, while the other branch presents a most degenerate class. The degenerate branch of the Kallikaks had produced to the time of Dr. Goddard's publication in 1912, 1146 individuals, 262 of whom were feebleminded, 197 abnormal and the rest still undetermined. Alcoholism and prostitution are very common. This condition was considered by Goddard to be transmitted as a simple Mendelian recessive, *i.e.*, the mating of two feebleminded people would give feebleminded offspring. This was modified by Danielson and Davenport in their report of the Hill Folk in 1912. They considered feeblemindedness as being more of a legal or sociological term and according as to whether the individual possessed a certain set of socially important traits he was either feebleminded or not. So if we mated two people with different kinds of feeblemindedness, *i.e.*, people who lacked different sets of socially important traits, we would get some normal offspring.

It has been claimed by Heron and Pearson that feeblemindedness is not inherited in the Mendelian way because we get all gradations from lowest forms of idiots to high grade morons. This does not follow, for the variation is one of degree and not of kind—so that we get different somatic modifications resulting.

Very closely allied to feeblemindedness is hereditary epilepsy. How it is related to the inheritance of feeblemindednss is not known, but epileptic offspring occur when two feebleminded people are mated. So the conclusion that it is inherited as a simple Mendelian recessive is not adequate. Probably as Davenport and Weeks pointed out, epilepsy and feeblemindedness are due to "the absence of a protoplasmic factor that determines complete nervous development and that the condition is recessive to the normal."

Even less clear is the case of insanity. In the first place, there is a great controversy over the percentage due to inheritance and the percentage to environmental influences, as diseases, injury, severe mental shock, worry, childbirth, syphilis and alcoholism. The percentages vary from ten to ninety per cent, but the concensus of opinion among modern writers seems to be that insanity is largely inherited. Since it does not always become manifest until after the period of adolescence and often during middle age and old age, the way in which it is transmitted is harder to follow. There are so many different forms of insanity, that we cannot speak of its being inherited as a unit character due to defect or loss of a single character in the germ plasm for that woud be "on par with ascribing all kinds of heritable physical anomalies to the same cause." Rosanoff and Orr who made the first serious attempt to study insanity from the point of view of Mendelism, came to the conclusion that it behaves as a Mendelian recessive character but they claimed that various neurophathic taints dominant to the normal may be recessive to other taints. This would make the Mendelian formula more elastic and increase the difficulty of proving the inheritance Mendelian. This Holmes thinks is probably the best explanation, so far, of the mechanism of the inheritance of mental deficiencies, for he says, "Imperfect dominance is sufficiently plentiful among organisms in general to make us expect it more or less frequently in the inheritance of neuropathic traits."

Associated with these characters is the inheritance of the state of mind which leads one to become a criminal. It is true that the environment has a great deal to do with the turning out of the ultimate criminal, but we must not forget the equipment with which he is thrust into his environment. Through studies of families of criminals it can be easily seen that there is always some neuropathic taint which runs in the family and manifests itself in many different ways. Though the theories of the positive school that born criminals are ataval reversions to the ferocious lower beasts have been repudiated, much of what appears to be atavism may result from arrested development due to diverse pathological causes. One of the most enlightening works on the subject is the history of the Juke family begun by Mr. Dugdale and completed by Dr. Eastbrook. Cases of prostitution, pauperism, criminality and other forms of degeneration, are very common. The born criminal is very often epileptic or feebleminded. Though nothing definite can be known concerning such a complex character, no one will deny that it rests on a fundamental basis of inherited defect of some sort.

Since it has been shown that defective mental traits are inherited in some way, possibly Mendelian, can it also be shown to be the case in the inheritance

of superior mental ability? From the extensive work done by Galton on genealogies of famous men we can draw the conclusion that superior mental ability is inherited. But how is very difficult to ascertain since mental traits, as has been seen, do not present the sharply definable and discreet features often characterizing the physical peculiarities of the body. Davenport and Hurst, from their investigations, place certain intellectual accomplishments in the category of recessive Mendelian characters, but others think that it behaves as a Mendelian dominant to inferior mental ability.

The great role played by environment or the existing social order must not be forgotten, when the subject of greatness is discussed. No one will deny that many men have died unknown for the lack of the proper environment which would have brought out their particular genius. The fact that inventions and scientific conclusions are invariably arrived at by more than one person independently at the same time is sufficient proof that social evolution has reached the stage where that particular thing, whether object or idea, was to be born.

From the study of the inheritance of mental traits, the great problem facing modern society becomes very evident. Is it because of sentiment or insufficient education that we keep on fostering the feeble-minded and delinquents, allowing them to propagate at their own free will and then supporting them afterwards by contributing great sums which might be much more profitably spent on education? Mr. Dugdale estimated that the Jukes have cost the state over $1,308,000.00 up to 1902. What is to be done about this? Are we to go on or are we to come out and speak for a purer and more efficient race? That is the task of the Eugenist and can only be arrived at through the popularization of the results of the investigations on heredity.

Traits Shown to Be Inheritable in Man

Dominant

Congenital cataract—condition of opacity of lens of eye which produces partial or total blindness.

Retinitis pigmentosa—pigmentary degeneration of retina also involves atrophy of the optic nerve.

Hereditary night-blindness (hemeralopia)—only can see in brightest light.

Achondroplasy—abnormally short limbs with normal head and body.

Keratosis—thickening of epidermis.

Epidermalysis—excessive formation of blisters.

Hypatrichosis—hairless, toothless condition.

Diabetes insipidus.

Diabetes mellitus.

Ordinary (not Gower's) muscular atrophy.

Glaucoma—internal swelling and pressure of eye-ball.

Displased lens.

Coloboma—open suture in the iris.

Spottedness of hair coat.

Corneal opacity.

Huntington's chorea.

Recessives

Albinism.

Certain forms of insanity.

Hereditary feeble-mindedness.

Chorea (St. Vitus' dance).
True dwarfism.
Alkaptonuria (urine darkens after passage).
Alcoholism and criminality where based on mental deficiency.
Hereditary hysteria.
Multiple sclerosis (diffuse degeneration of nervous tissue).
Friedrich's disease (degeneration of upper part of spinal chord).
Merriere's disease (dizziness and roaring in ears).
Thomsen's disease (lack of muscular tone).
Hereditary ataxia.
Tendency to become hard of hearing with increased age.
Non-resistance to tuberculosis.

Sex-Linked

Color blindness.
Haemaphilia.
Nearsightedness (sometimes).
Neuritis optica (progressive atrophy of optic nerve).
Gower's muscular atrophy.
Some forms of night blindness.
Ichthyosis—peculiar scaly condition of skin in some cases.

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STRUCTURE, DEVELOPMENT, AND FUNCTION OF HEMOLYMPH GLANDS

THE hemolymph glands have never been the object of intensive research. Lately, however, they have gained increased significance due to their extraordinary hemolytic action and their compensatory reaction after splenectomy.

I. Structure

The structure of a hemolymph gland resembles in several respects that of the ordinary lymph gland. It has a capsule of fibro-elastic tissue with a few unstriped muscle fibres; trabeculae of connective tissue extend from the capsule into the node dividing it into nodules; fine strands of connective tissue are then given off which enter the nodules and form a dense syncytial network—the supporting structure of node. Finer strands of connective tissue extend into the blood sinuses and are instrumental in retarding the flow of blood. Lymphoid tissue is also present either in large masses nearly filling the entire node, or as small patches found here and there. Cortical nodules with germinal centers where active mitosis is going on may also be present. Here and there amongst the lymphocytic mass are seen large mononuclears, polymorphs, taking all three stains, and rarely a megakryocyte or a polykaryocyte cell. Some investigators would also include nucleated red blood cells in this list.

The large mononuclears are especially interesting, since a great number of them both within the blood sinuses and among the lmphoid mass are filled with an ingested erythrocytic content. Their nature and significance will be discussed more fully under the heading of the functions of the hemolymph glands. The mononuclear easinophiles described by some observers as being present, are probably the phagocytic mononuclears with eosin stain. Nucleated red blood cells have also been identified by some in the interior of the large mononuclear cells. Meek states that he has observed in the hemolympth gland of the pig (in the human but very rarely), in their blood sinuses, nucleated red blood cells. In listing the cells of the hemolymph gland, the stellate cell of the connective tissue must also be mentioned since it has been said to be an important phagoytic agent of the gland.

Afferent and efferent lymph vessels and intranodal lymph sinuses may or may not be present. The intranodal lymph sinuses may or may not communicate with the blood sinuses. It has already become known to you that the main difference between the lymph gland and hemolymph gland is that in the latter the subcapsular sinues and the intradodal sinues are filled with erythrocytes instead of lymph. The subcapsular sinues is a space separating in a crude, irregular, manner the periphery of the lymphoid mass from the connective tissue capsule. The smaller intranodal sinuses also lie between the lymphoid masses and the strands of connective tissue penetrating into the

interior of the node; they communicate with the subcapsular sinus. Our data points to the fact that the sinuses are lined by endothelium, which is nothing more than a continuation of the capillary wall. Other views held are that the sinuses are lined by a fenestrated syncytial structure or that they have no lining at all; *i.e.,* they are cavernous and are surrounded on all sides by the lymphoid mass.

The circulation within the node of the blood, from its entrance by afferents, to its exit by efferents, is somewhat as follows: The number of small arteries entering the hemolymph gland varies. Several may pierce the capsule or only one may enter the gland through the connective tissue at the hilum. The afferent artery follows the course of the trabeculae, becoming smaller and smaller in diameter; when it has reached the periphery of the lymphoid mass it dilates to become the subcapsular sinus; smaller dilated sinuses are given off from the large sinus which enter the interior of the node. The medullary blood sinuses are therefore the terminations of the arterial capillaries and the beginning of the venule capillaries. From these sinuses, venules are formed which unite to form the vein which leaves at the hilum. Because of the dilated condition of the capillary walls the blood sluggishly percolates through the sinuses, taking up at the same time leucocytes and waste material. Lewis and others have observed normoblasts within the blood sinuses; this, however, is still doubtful.

Warthin, although he does not regard his classification of H. L. N. as absolutely correct, believes that the one he proposes is the clearest possible under the circumstances. Thus, he would call the glands under discussion hemolytic glands because of their hemolytic function and further subdivide them as follows:

1. With blood sinuses only.
   A. (Splenolymph).
   B. (Marrowlymph).
2. With blood and lymph sinuses not communicating (hemolymphatic).
3. With blood and lymph sinuses communicating within the node (hemolymph).

Before concluding the statements concerning the structure of the hemolymph glands it would be advisable in order to bridge over the gap between the structure and development, to note the presence in some of them of a tissue which presents itself perhaps as evidence that it was the forerunner of the hemolymph gland. This is adipose tissue.

II. Development of Hemolymph Glands

The two ideas concerning the development of hemolymph glands are:

1. Are hemolymph glands organs sui generis and if so from what are they developed? and

2. Are hemolymph glands developed from lymph glands and if so in what manner? In this connection the theories advanced by the four investigators, Warthin, Meyer, Drummond and Sabin will be taken up in order.

(1.) Warthin, who has done more constructive work on the study of hemolymph glands than any one else states that these glands are organs sui generis and are developed in adipose tissue in the following manner. In the prevertebral fat there appear certain fat lobules which stand out from the

rest in having a more definite capsule of connective tissue and a richer vascular supply. After splenectomy he noticed that the intralobular fat capillaries became dilated so as to form a small sinus. The bordering fat cells lost their adipose inclusion and became converted into reticular connective tissue cells which surrounded the capillary. Lymphocytes passed by diapedesis from the capillary into the reticular mesh-work, and there multiplied. By further fat absorption and sinus formation the structure of an adult hemolymph node was produced, the peripheral and central blood sinuses being formed by the dilated capillaries. If later on the lymphocytic production was very great, then the diameter of the capillaries was reduced greatly in size, and an ordinary lymph gland was produced.

(2.) Meyer, another intensive research worker on hemolymph glands, gives the following description of the formation of hemolymph nodes from the mesenchymal tissue of the embryo. In a subcutaneous portion of the fetus he found first a syncytial structure, condensations of the mesenchymal tissue. The cell outlines of the syncytial mass cannot yet be made out. Around this condensed mesenchymal portion appears a space which has no connection with any part as yet of the vascular system. Outside of this space there appears another circular region of condensed mesenchyme which represents the anlage of the capsule of the mature node. No capillaries or erythrocytes have yet appeared. Later on a few distinct polygonal cells can be seen in the center of the mesenchymal mass and as time goes on cell outlines become more and more distinct. The mesenchymal differentiation did not take place in or among a plexus of blood vessels. At a little later stage there appears in the perinodal space numerous erythrocytes and lymphocytes. Extra-nodal capillaries and arterioles penetrate the perinodal condensation of mesenchyme, enter the perinodal space and sometimes the node itself. The fetal node now resembles an adult hemolymph node except for the absence of a capsule. The peripheral blood space is irregular and has invaginations both into the node and into the surrounding mesenchyme, simulating the appearance of blood vessels. Later on a capsule is formed from the surrounding condensed mesenchyme and the erythrocytes may penetrate into the parenchyma mingling with the lymphocytes. Larger arterioles now penetrate the capsule, entering the perinodal space and also the node. Cellular differentiation has proceeded apace but no lymph follicles are present as well as germinal centers. The typical cells now are the lymphocytes which appear in thickened masses at the periphery of the node extending into the perinodal space which seems to have no definite boundary. The earliest vascular relations are established by the advent of capillaries and arterioles from the surrounding tissues into the perinodal space and node.

(3.) Drummond's idea of the development of the lemolymph gland is that they are organs sui generis and develop in the parenchyma cells of the mesenchyme from or about a lymphatic plexus. He is opposed to the idea of their development from lymph nodes in spite of the fact that they closely resemble them in many points of structure for the following reasons:

(A) Hemolymph nodes are not found in places where lymph nodes are found.

(B) Some hemolymph nodes develop in the mesenchyme before lymph nodes.

(C) The gap in structure between the two glands is too wide.

The development in detail as worked out by the above investigator is as follows:

In its early stage is is similar to the development of lymph nodes. Special cells in the mesenchymal mass become flattened and opposed to one another, forming a plexus of lymphatic vessels. Close to this plexus the first capillaries with their erythrocytic and leucocytic content appear. The lymphocytes pass by diapedesis from the capillary wall of the blood vessel out to the embryonal mesenchyme and are caught in the meshes of the lymphatic plexus. Here they proliferate rapidly, forming a lymph nodule. At a later stage it was found that a capsule had been formed around the lymphoid mass by a thickening of the mesenchymal connective tissue. In the interior of this young gland a fiberous stroma formed a syncytial meshwork acting as a supporting structure. Beneath the capsule great numbers of erythrocytes and leucocytes then appeared and many red cells were apparent in the meshes between the lymphoid masses.

It is easily seen that the final description given above is a picture of an adult hemolymph node. The blood capillaries, according to Drummond, were also developed from the mesenchyme and grew around the lymphoid mass after penetrating the fibrous capsule.

(4.) Sabin's statement of the development of hemolymph glands is that it is the same as that for lymph gland, except that in the former we have the lymphocytes surrounding a plexus of blood capillaries and a peripheral sinus of blood, while in the latter we have a plexus of lymphatics forming the basis of the development of a lymph gland.

The above descriptions point to the fact that hemolymph glands are organs sui generis. Other investigators, however, claim that hemolymph nodes are formed from lymph nodes and vice versa, depending upon the physiological condition of the body; in disease, for example, when a great number of erythrocytes are being destroyed there is a change from lymph to hemolymph glands by an angiectatic dilation of the capillaries in the gland and a degeneration of the extranodal lymph vessels. In a change from a hemolymph to a lymph gland there is a very rapid proliferation of lymphocytes with a corresponding diminution in the size of the capillaries and obliteration of the blood sinuses. Meyer and Drummond have both given reasons for the disapproval of such a theory as the conversion of a mature lymph node into a hemolymph node and a summary of our data would indicate that the hemolymph organs arise sui generis.

III. Function of Hemolymph Glands.

Of the functions of hemolymph nodes the data at hand allows of a division into formative and destructive activities. The destructive functions of these structures are undoubtedly their outstanding feature and as such will be considered first.

1. *Destructive Functions*

Early in the course of their investigations research workers were struck with the presence in the blood sinuses of the hemolymph glands of large cells containing in their interior pale bodies which in many instances were stained with eosin. Two interpretations were possible of this phenomenon; the first was that the pale bodies contained in the interior of the peculiar large cells of the hemolymph organs were nothing more than red blood corpuscles in

the process of formation; the second, just the opposite of the first, maintained that these large cells had taken up red blood corpuscles into their interior by phagocytosis, there to undergo disintegration. It is obvious that if the one view be carried to its logical end there will be assigned to hemolymph glands the function of producing red blood corpuscles, while if the second and opposite view be carried to its logical end there will be assigned to the hemolymph glands the function of the destruction of red blood cells. The second view, that the cells containing what seemed to some to be erythrocytes, are in reality only phagocytic cells, appears to be amply supported by one data and will be discussed under the present heading of "the destruction of red blood cells"; the first view will be taken up later under the head of the "formative functions of the hemolymph glands."

"Phagocytosis of the red blood corpuscles is the most striking feature of the hemolymph glands," writes one investigator, "and has long been recognized as one of their more important functions." Its extent varies greatly in individual cases of the same disease and in different glands from the same individual. However, all the various phases of the disintegration of the red blood corpuscles while contained in the phagocytic cells can be readily made out. In the interior of the phagocytes the injested erythrocytes may retain their normal appearance and staining reaction for some time; gradually, however, they swell slightly, and lose their property of staining with eosin, and finally appear like small vacuoles in the protoplasm of the phagocyte. These are commonly clustered around the nucleus, the peripheal part of the protoplasm being free from inclusions. Soon after, this pigment begins to appear in the peripheal part of the protoplasm in the form of minute granules of a golden yellow color. Whether the red corpuscles are completely digested or whether any "membrane" is extruded from these cells, it is difficult to say; such membranes have been described by Vincent and Harrison. The pigment thus deposited appears to run together into larger, irregular, homogeneous masses, which may almost entirely fill the protoplasm of the cell. Such masses of pigment, either contained in cells, or free, may become so abundant as almost to block up some of sinuses.

The destruction of red blood cells as it is carried out by the hemolymph gland, seems to have some limiting factor and one may cautiously suggest that it is an intermittent or cyclical function as contrasted with a continually performed duty. An examination of several hemolymph glands which may be taken from the same source will well serve to bear out this point of view. For example, in one particular specimen one may find that the sinuses are filled with red blood cells, amongst which are a considerable number of leucocytes, but careful search may fail to reveal a single cell with red corpuscles in its protoplasm. In another gland phagocytic cells may be quite numerous in practically all of the sinuses and in practically all of which red blood corpuscles at about the same stage of disintegration can be seen. Again, in specimens where pigment is abundant, all of the pigment may be in the form of small granules or all of it may be in the form of large masses. These facts seem to indicate a cyclical function on the part of the individual glands. What starts this process is not known, but as suggested by Drummond, it may certainly be supposed that once started, the number of phagocytic cells in the sinuses increases rapidly and at the same time individual cells attack and engulf the red corpuscles which are abundantly present. By this double

process of the increase of the number of phagocytic cells and increase in their individual size, the sinuses soon come to be completely blocked by them. Circulation through the sinuses is now at a standstill, because nearly all of the red blood corpuscles in the sinuses have been engulfed by the phagocytes. If this process takes place with some degree of rapidity one can understand how it is that we sometimes find a gland containing thousands of these cells in every section, every one of these containing apparently unaltered red blood corpuscles. Thus if we were to carry this mechanical explanation of the periodic phenomenon of phagocytosis in the hemolymph glands, as proposed by Drummond, only one step further, the logical conclusion could be arrived at, that the different phases in the disintegration of the red blood cells, having begun together, will run a parallel course.

That the number of the hemolymph glands depends upon the rate of phagocytosis as it takes place in the individual glands is well illustrated by an examination of the number of hemolymph glands of the sheep, ox, dog, and cat. In the first two, the sheep and ox, the rate of destruction being minimum in the individual glands, there are thousands of these present, while, however, in the second two, dog and cat, where the rate of destruction in the individual glands is maximum, the glands are actually numbered by units. A little thought will undoubtedly give rise to the question that in the case of those animals where the rate of destruction is maximum will there not be a quick choking up of the sinuses with pigment and a consequent delayed circulation. It is of interest to note in this connection that another important difference between the glands of the dog and sheep is the remarkable development of the arterial supply of the former as compared with the latter. It is this very great development of the arterial system of the dog's hemolymph nodes that accounts for the ability of these blood-destroying structures to continue their processes in spite of blood sinuses literally choked with thousands of phagocytic cells contains their prey. As a corollary to these observations one may add the conclusion that the development of the arterial system of the hemolymph node in general is associated with the difficulty of maintaining the circulation through the gland.

This question is now brought up by Lewis: is phagocytosis or intracellular destruction of erythrocytes the only means by which erythrocytes are destroyed? Is it not also possible that the phagocytes in addition to enclosing and digesting the red blood corpuscles, have also the power of secreting substances of a ferment nature which are capable of acting on erythrocytes or other blood corpuscles? According to Lewis, in certain glands of the Ungulata, in many of the sinuses, the blood corpuscles, more especially the erythrocytes show marked evidence of disintregation. The causes of the appearances of disintegration is by no means clear. The hypothesis of Lewis was, however, not put on any firmer basis by the work of Meek who experimented with gland fluid in an effort to see whether or not the gland cells or gland tissue secreted a substance with hemolytic powers. His experiments rather favored the view that the phagocytosis of erythrocytes was independent of any specific substance formed by the tissue proper.

2. *Formative Functions.*

A. To repeat what has already been said the first interpretation of the pale bodies contained in the large peculiar cells of the hemolymph nodes was that they were red blood corpuscles in the process of formation (so described

by Robertson, Clarkson, and Gütig.) If such is the case, then the hemolymph nodes can certainly be said to be a source of red blood cells just as the red bone marrow is. However, most of the investigators of the problem agree that under normal conditions the hemolymph nodes do not show such a function as the formation of erythrocytes. Warthin does make the statement that while he has seen nucleated red blood cells under pathological conditions he has never seen any in normal nodes.

Though time has abandoned the old view of the origin of erythrocytes from what are now definitely known to be phagocytic cells, one investigator, namely, Meek, still holds on to the idea of the formation of red blood cells in the hemolymph gland. His proof of this formation is however not based on an origin from the pale eosin staining bodies of the phagocytes, but upon observations which he made of such glands in the pig and human in which he claims to have seen and pictures nucleated red blood cells. The uniqueness of his work and his solitary position with reference to this function of the hemolymph glands demands our close attention.

"Of especial interest," says Meek, "are certain islets of cells which occur in the midst of the blood in the sinuses. They are sharply defined from the surrounding red blood corpuscles, and one such focus will contain perhaps 50 closely packed normoblasts, another a lump of myelocytes, neutrophilic or eosinophilic in granulation, while yet others are made up of aggregations of polymorphnuclear cells. Mitotic figures may be seen in the cells of these islets. Another cell of interest to be seen in the sinuses, though only occasionally, is a large cell closely resembling the megakaryocyte of bone marrow." The presence of these nucleated red blood cells would seem to suggest that a part at least of the function of these glands in the pig, where a large number of them are easily seen, is connected with the formation of red blood cells. However, the same author, in the same article, states that glands examined by him taken from patients dying with signs of grave anemia very rarely showed the occurrence of nucleated red blood cells. Again no instance was met with in which nucleated red blood cells were found in appreciable numbers in the hemolymph glands without the presence of these cells in considerable number in the bone marrow and blood. These findings somewhat confirm the belief that the hemolymph glands may assist or even replace the bone marrow (though the latter is hardly possible) insofar as this particular function is concerned. The conclusion Meek draws from his experiments is that histological evidences of the hemopoietc function of the hemolymph glands are only to be seen in man in comparatively rare instances, and that when exercised the reaction is just as a rule secondary to a similar action of the bone marrow. Just as Meek stands alone in his observation of nucleated red blood cells being present in the sinuses of the hemolymph nodes so does Retterer, who holds still another view of the formation of red blood cells within the glands, namely, that they arise from the lymphocytes by a hemoglobin transformation of the nucleus, remain unconfirmed in his work. Thus, according to Meek, the production of nucleated red blood cells in the glands of the pig undoubtedly takes place as shown by their presence under the microscope and a similar production in human hemolymph glands is not so well marked but is rather dependent on the reaction of the red bone marrow in its formation of red blood cells.

B. The constant presence of lymphoid tissue more or less abundant, the usual presence of germ centers in which cell division is actively going on,

indicate that the hemolymph glands serve as active centers for the manufacture of leucocytes. The arrangement of the blood sinuses around the lymphoid tissue is extremely favorable for the rapid escape of the leucocytes into the general circulation.

C. Polykaryocytes and some megakaryocytes are found in the nodes. These, to be sure, could not easily leave the node or be transported there because their great size and must hence have arisen within the node itself or else have been transformed there.

D. An increase in the number of endothelial cells is one of the most constant morbid changes seen in hemolymph glands. These cells are familiar as the main phayocytes of lymphoid tissue and play a prominent part in the destruction of red blood corpuscles. Their phagocytic activity is often very great and an individual cell may be seen to contain 20-30 red blood corpuscles.

E. The polymorphnuclears and neutrophile myelocytes seen in the hemolymph glands by Meek were confined almost entirely to the sinuses, and the appearances suggested that the sinuses were probably acting as a site for their production or multiplication.

F. Of especial interest are the presence in the glands of numerous cells containing coarse granules strongly stainable with eosin. In the majority of these cells these granules are closely packed; in other cells they are large and more irregular fragments; in a very few an entire erythrocyte is included. In some of the sinuses eosinophile cells are seen in contact with damaged erythrocytes or surrounded by debris already described as consisting of eosinophile granules. In those cells in which few granules are found, the ring of protoplasm surrounding the nucleus is small, and the latter is almost always singular, such cells approach in size and appearance the well known lymphocyte. The question may now be asked: Do not these appearances suggest the formation of eosinophile leucocytes from the products of disintregation of the red blood cells? It at first sight appears unlikely that a small uninucleated cell should develop into a far larger one with not a round, but a polymorph nucleus. The injection of numerous particles, however, might be a potent factor both in the distension of the cell wall and in the distension of the nucleus.

3. *Compensation for Spleen.*

While it is not the purpose of this paper to give a complete discussion of the function of the spleen we thought it would be useful, since this organ is often classed as one of the hemolymph glands, to draw a comparison between the two, and particularly to see what changes the hemolymph nodes undergo in cases of spleenectony or absence of the spleen. Thus, the conclusion might be reached that the hemolymph glands and spleen are closely allied and have the same main activities in the body, if it can be shown that the hemolymph organs can replace the spleen in the case of the latter's absence.

Due to the general similarity in structure of the hemolymph glands and spleen, their common function of hematolysis and the occurrence of transition forms resembling accessory spleens, but chiefly due to the autopsy findings in a case of spleenic anemia in which there was throughout the mesentery fat a new formation of hemolymph nodes resembling spleenic tissue, Scott Warthin, the author of the series of experiments upon which this part of our article is based, put forth the hypothesis that hemolymph nodes under certain pathological conditions may compensate for the spleen.

Spleenectomy was carefully performed on eight animals; six sheep and two goats were used in these experiments; mechanical difficulties leading to injuries were avoided as much as possible during the operations and fairly normal results were obtained. All of the animals recovered soon after spleenectomy and were carefully examined at stated intervals of one week, two weeks, one month, two months, and five months, with particular references to the changes in the hemolymph nodes which they possessed. The results are given in the following table:

Time	Phayocytic Activity	Proliferation Lymphoid Tissue	New Formation of Hemolymph Nodes
1 Week	Increase; shown by increase in phayocytes and number of cells containing eosinophiles granules.	Beginning of Proliferation.	
2 Week	Greatly increased; shown by same facts as 1, only in greater amount.	Active proliferation; evident by numerous mitoses present throughout hemolymph node.	
1 Month	More marked increase.	More marked proliferation.	New formation. Hemolymph node in adip. tissue.
2 Months	Increased Hemolysis: Marked eosinouhilia.	Advanced hyperplasia of all lymphoid structures.	New formation in adip. tissue.
5 Months	Marked Act: Eosinophilia.	Great hyperplasia.	New formation in adip. tissue.

It is evident from the preceding table that spleenectomy in the sheep is followed during the first five months by a compensating hyperplasia of preexisting lymphoid tissues, transformation of hemolymph nodes into ordinary lymphatic glands, and a new formation of hemolymph nodes in the adipose tissues. No evidence of regeneration or new formation of spleenic tissue was found; likewise no evidence of formation of red blood cells in the hemolymphatic glands after spleenectomy was found. Insofar as the question of compensation for spleenic function is concerned, the results of the above experiments would bring out the fact that destruction of red blood corpuscles and leucocyte formation are the two functions which are taken up to an increased degree by the hemolymph nodes and lymph glands after spleenectomy. That the spleenic function is not entirely compensated for is shown by the disturbed equilibrium of the blood in the excess of hemolysis over blood formation. According to Warthin, this might be explained by the hyopthesis that there is some hemolytic agent found in the body which is normally taken care of by the spleen or the spleen has some influence in the new formation of hemoglobin.

Several other facts brought forward by S. Warthin are also of some interest. The new formation of hemolymph nodes along the hilum or along the vessels of the peripheal glands whose medullary portion showed pigmen-

tation, might be taken, according to Scott Warthin, in support of the view that the pigment is carried from the neighboring hemolymph node to the lymphatic glands through the lymphatics. The hemolymph nodes would hence be acting as hemolytic organs passing their products of blood destruction on to the lymphatic glands for further elaboration. . The presence after spleenectomy of a great number of eosinophiles in those glands of the experimental animals showing great destruction of red cells seems to point to some relationship between these cells and the destruction of the latter; one would not be stretching it very far to come to the already spoken of conclusion that eosinophiles arise directly from the disintregation products of the red blood cells being taken up by the small lymphocytic cell.

As far as could be determined, no definite function as regards the general immunity of the body and the origin of antibodies was assigned by investigators, as a result of their experiments, to the hemolymph nodes. However, several of these men have hinted in their papers that these glands might play an important part in the production of immunity and the protection of the body through the formation of antibodies. When one considers the close resemblance of the hemolymph nodes to the spleen, which organ is fairly definitely established as a center of origin for antibodies, or at least of an anti-hemolytic agent; also when one considers the close connection of the cellular make-up of the hemolymph glands with that of the blood, the hyopthesis that they may produce anti-bodies does not seem so far fetched.

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SENESCENCE AND REJUVENESCENCE

IT is proposed in this paper to give a brief outline of some of the aspects of the problem of Senescence and Rejuvenescence. It has been necessary to leave out considerable data and in going over the available material it seemed possible to bring out the main points of the problem while omitting the work on plants and that on the specific diseases of old age. The discussion has therefore been limited to four main topics, histological and morphological changes in senescence and rejuvenescence, physiological changes; some of the present day theories of senescence and rejuvenescence, and some of the experiments.

The problem of senescence and rejuvenescence is essentially one of living matter, that is of the living organism. But what is the organism? There are many theories, the neo-vitalistic, the corpuscular, the chemical, the physiochemical and the colloidal substratum of the organism, are a few of them. C. M. Child defines the organism as: "A specific complex of the dynamic changes occurring in a specific colloid substratum which is itself a product of such changes and which influences their course and character and is altered by them." He maintains that the differentiation and dedifferentiation of the organism is the basis of senescence and rejuvenescence. Senescence is, therefore, according to his definition, "a decrease in the rate of dynamic processes conditioned by the accumulation, differentiation and other associated changes of the material of the colloid substratum, and rejuvenescence is an increase in the rate of dynamic processes conditioned by the changes in the colloid substratum in reduction and dedifferentiation."

These definitions give us some idea of what is meant by senescence, but to show what old age really consists of we must consider the changes, histological, morphological and physiological associated with it.

"Old age," as a term usually brings to our minds a definite picture. We are familiar with the shrunken stature, the shuffling gait, the wrinkled face and the slow mind of the old man of the human species. The decrease in stature is always very marked and is due largely to the bony fusion of the spinal column. It was estimated in some German statistics that though at thirty the average height for man was 174 centimeters, at seventy the average was only 161. It is well known also that the long bones of the old break more easily than do those of the young, due to a smaller amount of spongy or cancellous bone and a greater amount of solid bone. This increase of bone tissue is seen everywhere, especially in the cartilage of the ribs. It is progress of a kind, but progress carried to excess. The shuffling gait is largely explained histologically by an actual decrease in the number of muscle fibres in a given muscle. They apparently wear out. The wrinkled appearance of the skin is merely indicative of what is going on within, and this process is most generally given the specific name of senile atrophy.

This phenomenon, if such it may be called, of senile atrophy, concerns practically all the more highly specialized organs and to some extent all the tissues in the body. It is more noticeable in the higher forms of life which

have more highly specialized tissues. The changes of senile atrophy consist essentially of a decrease in the size of the cell and more or less degeneration. It must not be assumed that all tissues are simultaneously affected by senile atrophy for it is obvious if this condition exists in a certain proportion of the tissues, death will occur. This atrophy of the tissues is undoubtedly associated with the decrease in the rate of metabolism, which will be discussed under the physiological changes of senescence. This shrinkage in old age would be much more marked than it is, were it not, that as the more highly diffnrentiated tissues give way to senile atrophy, they are, at least partially, replaced by connective tissue. As Wooton puts it, "Histologically death from old age is the victory of connective tissue over the more specialized tissues."

One of the places where this senile atrophy has been most observed is in the brain and nerve cells, and this relates itself, of course, to the enfeebled mentalities of the aged which was noted in the beginning as one of the most obvious outward signs of age. Perhaps the most distinguished piece of work in this line was done a good many years ago by Hodges on the changes in the ganglion cells from birth to senile death in many and honey bees. In man, he made careful, though not exhaustive, examinations of two sets of ganglion cells, one set from an old man dying from senile decay at the age of ninety-two and the other from a new born infant. In the former, the cytoplasm was full of pigment, the nucleus shrunken and irregular and the nucleolus poor staining, whereas in the young ganglionic cells the cytoplasm had little pigment, the nucleus was round, and the nucleolus deep staining. His experiments on the honey bees, carried on at the same time, afforded a chance for more control and for a larger number of subjects. The results tallied very well with his observations on the human. In the old, the cytoplasm was full of large granules, many vacuoles, and the nuclei were shrunken, whereas in the young the cytoplasm was evenly granular and the nuclei large and clear. These observations of Hodges show why it is so often noticed that the brain of the old is actually shrunken. In persons of a height of 175 cms. of male sex of twenty to forty years the brain weights 1,409 grams, from 41 to 70 years it has shrunken to 1,363, and from 71 to 90 years it has shrunken to 1,330 gms., on the average. The shrinkage begins soon after the attainment of maturity and continues steadily to the end of life.

Another important histological change, due to senile atrophy, is the change in the arterial system. The arterial system invariably shows changes in the direction of decreased elasticity and contractility of the artery walls resulting in arterio-sclerosis. (We will see later that all the authors do not feel that arterio-sclerosis is as fundamental a fact of old age as it was once believed to be.) Due to the changes in the arterial system, the heart often hypertrophies instead of atrophying, but this is generally believed to be a functional reaction due to the increased work of the heart, necessary because of changes in the arterial system.

Not all the changes of old age are morphological and histological however. Some can only be classed as physiological. The striking physiological fact is the decrease in the rate of metabolism. Aub and De Bois, to cite merely one of the experiments on the basal metabolism of the aged, carried on a careful series of experiments in 1917 on six old men between the ages of seventy-seven and eighty-three, with the calorimeter apparatus. Their average basal heat production was 35.1 calories per square meter per hour. This is twelve per cent lower than the average for men between the ages

of twenty and fifty. Just what this lowered rate of metabolism may mean is stated by Wooton: "Senile decay is auto-failure, it connotes metabolic impairment and consequent poisoning." Defective oxygenation and inability to carry off waste products go hand in hand with senile decay. What lies back of this decrease in the rate of metabolism is not very clear. Bue we are lead to suspect some changes in the thyroid which governs metabolism, and at the present there is a good deal of discussion on this point. Summarizing then the changes seen in age, it might be said that the most important morphological and histological change was senile atrophy of the specialized tissues with an increase in the amount of connective tissue, and on the physiological side, a decrease in the rate of basal metabolism.

Various workers observing these changes of old age which we have put forth above have stressed one phase or another and have evolved various theories accordingly. Minot, on the one hand, sees in senescence and death merely the natural end of cytomorphosis; while on the other hand, according to the medical view, senescence is almost a pathological condition. The changes which particularly interested Minot are the histological changes of the cells. "Age," he says, "is the result of cumulative cytomorphosis." Senescence depends on the increase of cytoplasm in proportion to the size of the nucleus, together with its increased differentiation. The power of rejuvenation is lost when the nucleus becomes small in proportion to the amount of cytoplasm. There is no doubt that this relation is born out in observations on "young" and "old" cells, but as to whether cytomorphosis tells the whole story or whether it is merely an expression of the other factors working to produce senescence it is hard to say.

More closely related to the physiological changes but taking in only a very narrow field are Metchinokiff's theories. To him senescence was a result of auto poisoning produced by the bacteria of the large intestine. This is mentioned because of the wide publicity his theories have received but it does not cover all the ground.

There are those however, who in recent years have seen in the endocrine glands the answer to the problem. We have noted before that the thyroid and other endocrine organs, secondarily, may have a decided effect on the rate of metabolism. Lorand has pointed out that at a certain age the different ductless glands show important changes, notably an increase of connective tissue with consequent degeneration of the secreting tissue. Seventy thyroid glands which he examined in aged subjects showed marked degeneration. It is also seen that myxodemia and old age have certain points in common, which might lead to the conclusion that both were due to thyroid deficiency. So firmly do some believe this that they advocate the administering of thyroid extract in old age. These workers have changed the expression "A man is as old as his arteries" to read, "A man is as old as his endocrine glands." This theory is upheld by G. Stanley Hall in his recent publication on "Senescence." That all are not ready to accept this view is shown by an article, also recently published, by Thompson and Todd in the Lancet, in which they say that thyroid insufficiency is not characteristic of old age and that the simple wearing out of the mechanical factors of the organism from use is the more direct explanation.

It is regrettable as we review these theories to see that there is no one theory which by any means adequately takes into account all the changes of old age. The remedy will come we believe by the application of some

of the more carefully controlled experimental work on lower animals to the changes seen in man. For these reasons, bearing in mind the changes seen in man, some of the more recent experimental work as it applies to senescence and rejuvenescence will be considered.

Dealing with the lower animals, C. M. Child carried out a series of experiments on Planaria to determine the age differences in susceptibility. Using Planaria dorotocephala, Planaria maculata, and Planaria velata he concluded that there was a general age difference in the susceptibility of these animals to cyanides and narcotics. The younger animals showed the highest susceptibility and this susceptibility decreased with advancing development. In other words, the rate of metabolism is highest in the young animals of this species and decreases with advancing age. A similar decrease in the rate of metabolism with increase in age was pointed out in man.

Calkins in his experiments on Protozoa in 1904 used Paramoecia, which he cultivated in the laboratory. The Paramoecia, which were not allowed to conjugate, were observed to pass through cycles of vigor and depression, each cycle being of three months duration. During the depression period, division always slowed up and more accumulation of waste material occurred, different in kind in the later periods than in the earlier. This he maintained to be due to different physical conditions and to a weakening of different functions.

Although the conditions of the experiment were believed to be practically normal, death occurred after twenty-nine months, apparently from exhaustion, for at death there were no morphological signs of senility. The signs of old age seemed from this experiment to be functional, the slowing up of division, and the accumulation of more waste material in the later periods of depression, and old age seemed to be a natural condition of living protoplasm.

Other experiments on Paramoecia were undertaken by Woodruff, who has maintained a strain of these animals through two thousand generations. He observed that, "Their life history was characterized by a general decline of the division potent ending in death when the organisms were subjected to a constant environment but that the life history might be prolonged by the use of certain stimuli." He found, as did Calkins, that there was a periodic variation in the rate of multiplication, there being cycles of rise and fall of fission rate which ended in extinction unless conjugation occurred or there was a change in environment. No conjugation was allowed but, carrying on the experiment, frequent change in environment was made and the culture showed after forty-one months and two thousand generations that, "when subjeceted to suitable culture condition it, that is undifferentiated tissue, has the power of unlimited reproduction by division without conjugation or artificial stimuli."

Dr. Carrel carried on a series of experiments at the Rockefeller Institute in which he proposed to determine two things: first, whether a definite relation existed between the rate of multiplication of fibroblasts cultivated in plasma and the age of the animal from which the plasma was taken; and second, whether any modifications brought about by age in the action of the plasma on the fibroblasts were due to the disappearance of an accelerating factor or the production of an inhibiting factor in the plasma.

Using the technique employed in the cultivation of tissue in vitro and plasma from chickens, he tried a variety of types and ages of plasma. It was always found that the fibroblast rate of growth was greater in the

younger tissue. There was no doubt that the rate of multiplication of the fibroblast and the duration of their life in vitro varied in inverse ratio to the age of the animals from which the plasma was taken.

Various concentrations of the same plasma were used to find out whether it was the decrease in an accelerating factor or the increase in an inhibiting factor which caused the decrease in the growth in the fibroblasts cultivated in plasma of older chickens, and it was found that the serum of the three-year-old chick contained a factor inhibiting the growth of fibroblasts. At no age was high concentration found to be good, showing that there never was an accelerating factor present.

In still other experiments he fousd that by transfusing blood from a young dog to an old one a marked difference was soon observed in the physical condition of the old dog. He also tried a method of washing the blood to see if this inhibiting factor, if such it was, could be removed, and found that old dogs, some of whose blood had been removed, washed in a special manner, and reinjected showed distinct signs of rejuvenescence. These experiments have not as yet been completed and hence no conclusion can be drawn from them but they seem to point to the fact that senescence is due to the presence in the blood of a factor which inhibits growth.

With the exception of these experiments which are still incomplete, no work seems to have been done on rejuvenescence in higher animals. It appears that with high differentiation the power of rejuvenescence is lost to a greater or less degree, but in the lower animals in which there is a large amount of undifferentiated tissue this phenomena has been demonstrated.

Child in his experiments on Planaria produced new animals by reconstitution, these animals resembling a young animal in their morphological features and being capable of growth and development. He accomplished this by cutting pieces from Planaria; new embryonic tissue forming at the cut surfaces which gradually became old after its formation, while the other parts of the piece became young by reconstitution and reorganization until a dynamic equillibrium was established in the rate of metabolism in the different parts. He found that the rate of metabolism was higher in the cut pieces than in the same region of the body in an uninjured animal in the same physiological condition.

He concluded from these observations that, "The process of reconstruction brings about in some way a greater or less degree of rejuvenescence in these relatively simple animals and the degree of rejuvenescence is, in general, proportional to the degree of reorganization in the process of reconstitution of the piece as a whole."

By successive reconstitutions, alternating with feeding and growth, he brought the animals back to essentially the same stage in the age cycle in each successive generation, and by successive reconstitutions, without feeding and growth, the animals were made progressively younger physiologically in each generation until further reconstitution became impossible.

To sum up then, it seems that the problem of senescence and rejuvenescence goes back fundamentally to histological and physiological facts. The one fact common to all life is the physico-chemical relationship, for the change in the rate of metabolism observed from the experiments on lower animals is also seen in man. On the histological side, however, it has been pointed out that there is no such correlation between senescence in the lower and higher forms of life due perhaps to the greater differentiation of tissues

in the latter. Yet it is in the histological field that the most successful experimental work has been done on rejuvenescence, while we must look to the future for the solution of this problem in physiological terms.

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THE GROWTH OF TISSUES IN VITRO

WE owe to Harrison the first successful culture of tissues in vitro. In 1907 he was working on the problem of the origin of nerve fibres. Do nerve fibres grow out from ganglia, or do they differentiate from protoplasmic bridges? Harrison solved this long-standing embryological problem. But more significant than the solution of this problem was the introduction by Harrison of a new biological method. The reason why it had been previously impossible to come to a definite decision on the question of the origin of nerve fibres was that it had been impossible to separate the ganglia from the protoplasmic bridges. Harrison succeeded in growing isolated ganglia in vitro, that is, outside the organism. From a frog embryo, he obtained ganglia free from the surrounding tissue, and placed them in a drop of lymph from an adult frog, in a manner to be described later. In this lymph drop, he was able to observe nerve fibres growing out from the ganglia. Was this, however, a normal process? Do cells in vitro differentiate as they normally do in vivo, that is, in the organism? To determine this, cells whose normal differentiation was already definitely known, were put in lymph drops and observed. It was found that muscle plates differentiate into muscle fibres with cross striations, and that epidermis cells took on a cuticula and functioning cilia. There was, therefore, no reason to suppose that the outgrowth of nerve fibres from ganglia was an abnormality, due to in vitro conditions. On the contrary, this new technique enabled Harrison to conclusively solve the problem of the normal origin of nerve fibres.

Only a few years later another important biological problem was attached by the in vitro method. Can heart muscle contract without nervous stimulation? The weight of evidence in this much disputed question indicated that the rhythmical contraction of the heart is possible without nervous stimulation. Burrows contributed further evidence in favor of the myogenic theory. He isolated in vitro a small piece of heart muscle and observed that though unquestionably free from all nerve fibres, it beat with a perfectly normal rhythm, and with a rate and force similar to heart pulsation in the embryo.

The technique which Harrison and Burrows used was this: A drop of plasma (a culture medium which Burrows substituted for the lymph used by Harrison) is put on a cover glass, and on this drop is placed a minute fragment of the tissue. The cover glass is then inverted over a hollow slide so that the drop is suspended from the cover glass in the hollow. The actual technique is almost infinitely complex. The following are only some of the more important factors which must be considered. First: The tissue fragment must be very small, in general but a few mms. For every particular case, there is an optimum size, and if the fragment is the least bit larger or smaller than this the results are decidedly inferior, in fact, no growth at all may occur. Similarly the size of the drop is important, and there is in addition an optimum relation between the size of the tissue fragment and the sizes of the drop. Harrison used lymph as his culture medium, but Burrows

and Carrel found plasma more convenient. Furthermore, they found that plasma coming from the same species as the tissue fragment, that is, homogenous plasma gave better results than heterogenous plasma. The next improvement was the use of plasma from young rather than from adult animals. In all cases, after several days, death occurred because of the accumulation of metabolic products and the exhaustion of the food supply. To remedy this, cultures were removed from time to time to a fresh medium, after having been washed free from metabolic products with Ringer's solution. Even so, cultures did not survive more than several generations. By the addition of embryonic tissue juice to the medium, however, the life of tissue cultures could be prolonged practically indefinitely. Carrel, at the Rockefeller Institute, now has a strain of fibroblasts which has been growing in vitro for over ten years. The cells of this old strain have not changed their morphological characteristics since the first culture, and they proliferate at least as rapidly as the original fibroblasts. The plasma in the hanging drop soon clots. This is essential for some solid framework is needed for cell growth. If no clot forms, as in the case of amphibian plasma, where clotting is greatly delayed, there is no growth and Harrison proved that the fibrin functioned only as a framework by substituting for fibrin silk threads and spider webs. The protoplasmic bridges which were mentioned in the problem of the origin of nerve fibres, probably served as such a mechanical support for the nerve fibres as they grow out from the ganglia. We have described only a few of the necessary details of the technique. The most important precaution is to keep the culture free from bacterial infection. There are, however, a great many detailed precautions, the neglect of any one of which will result in the death of the cells. For instance, the exposure of thyroid tissue to the drying action of ordinary air for more than ten seconds kills the tissue. Indeed, according to Carrel, the entire procedure is best carried out in the humid atmosphere of the operating room. By means of this technique, it has been possible to grow in vitro almost all kinds of cells and tissue, leucocytes, epithelium, muscle, connective tissue, lymphoid tissue, cartilage, nervous tissue, tissue from kidney, heart, thyroid, etc.

For the present, then, so much for technique. What are the advantages of this method in studying biological problems? The value of studying isolated parts of the organism has long been recognized. In physiology, many discoveries have been made by experimenting on isolated organs, and the so called egg shakers of the school of Roux have advanced embryology by separating various parts of the embryo and observing the development of the separated parts. Even the idea of isolating tissues, and growing them in vitro, is by no means new. Leo Leob tried growing tissues in test tubes. In addition to the fact that he failed to get continued growth, there was also the difficulty of not being able to observe the tissues while growing. Harrison overcame this difficulty by adopting the hanging drop method of bacteriology. By this method, it is possible to observe the growth of isolated tissues in an environment which can be varied and controlled. The isolation of pure strains of tissues has been exceedingly difficult. So far there have been grown pure strains of connective tissue, epithelium, cartilage and leucocytes. Of these, all except the connective tissue were obtained only within the past year. Hence, it is probable that more pure strains will soon be obtained. Many of the early errors and uncertainties in work with tissue cultures can now be traced to the fact that the cultures were not pure. For example, a mass of ap-

parently sound experimental work was done on the transformation of epithelial into connective tissue. It now appears that this connective tissue had its origin in connective tissue already present in the culture. Had a pure strain of epithelium been used, this objection could not have been raised.

The method of tissue cultures has been mainly criticized on the ground that in vitro conditions are different from in vivo conditions, and that therefore it cannot be assumed that the processes observed in vitro are necessarily those whick go on in the organism. As Harrison has said, this same criticism might have been brought against the early science of chemistry by naturalists who claimed that chemists should concern themselves with descriptiive mineralogy rather than with abnormal compounds. The fact that conditions are abnormal does not mean that nothing can be learned from studying what occurs under the abnormal conditions. What can be learned is the reactions of tissues and cells to various definite environments? The total behavior of an organism is the complex result of the actions and interactions of the individual parts. In vitro, we are able to study the action of a part free from the complicating effects upon it of the rest of the organism. By this sort of analysis we can hope to build up a general science of a behavior of tissues and cells. Occasionally, by comparison with what is known to occur in the organism, one can find out whether a particular process observed in vitro is abnormal or not. For instance, as we have already said, Harrison observed that muscle plates happened to differentiate in vitro just as they were previously known to differentiate in the embryo. The fact that we cannot study in vitro the complex inter-actions which take place in the organism is no reason for avoiding the method of tissue cultures. The fetish of the organism as a whole, as Harrison put it, must not prevent experimentation in biology.

We now come to the results gained by the method of tissue cultures. These results often have very little connection with each other, for, after all, we are discussing a method rather than a problem. Investigators with all sorts of problems have used this metod of experimentation, and their results usually have no more in common than their problems. We shall use these varied results to illustrate the general advantages and possibilities of the method we are discussing. The advantages are briefly these: Isolation of parts, control of medium and the possibility of close and continued observation under the controlled conditions.

One type of problem studied was that of morphogenesis. We have already shown how Harrison demonstrated the origin of nerve fibres. In studying the genetic interrelationships of leucocytes and other cells, Maximoff and Carrel used the in vitro method with great success. Maximoff observed in vitro the transformation of lymphocytes into granular acidophil leucocytes. He could observe the steps of the transformation, a myelocyte, the normal ancestor of granular leucocytes being one of the definite stages. He also found that some lymphocytes and reticular cells became large mononuclears, and that some reticular cells became lymphocytes. More recently, Carrel found that some large mononuclears from a pure culture differentiated into normal fibroblasts (i. e., into primitive, connective tissue cells). This gave support to the old idea of Renaut that connective tissue is derived from lymphocytes. These experiments do not conclusively show that the transformations observed take place in the organism, but they do indicate certain intimate genetic relationships among various lymphocytes, granulocytes, large mononuclears, cells of

reticular tissue and fibroblasts, and they show the *possibility* of certain transformations taking place in the organism.

We shall now present several morphogenetic experiments which illustrate the convenience and reliability of the in vitro method. Richard Goldschmidt observed the whole course of spermatogenesis in vitro. The process of spermatogenesis, which takes weeks in the organism, can, by warming, be hastened in vitro so that it is completed in one day. McWhorter and Whipple were able to observe the continuous development of a blastoderm in vitro from a three to an eighteen somite stage. They found, as did Goldschmidt, that what they observed in vitro was, in all essentials, in agreement with previous knowledge. But certain things could be studied in vitro that either were difficult or impossible to study by the ordinary technique. For instance, they observed that on the collapse of a blood vessel the endothelium went back to undifferentiated mesenchyme. They also observed the formation of new blood channels, the first heart beat, and its immediate consequences. Since then, Sabin has used the same method to study the early development of the vascular system. These are only some of the applications of the in vitro method to the study of morphogenesis.

The most frequent application of the method has been in studies on the effects of different environments on the form and growth of cells. The form of the cell and the structure of the cell mass depend not only upon the tissue from which the cells were taken, but also upon the mechanical conditions of the medium—such as the viscosity of the drop, and the density of the fibrin network. A striking example of the effect of a medium on the form of the cell is seen in the change in the form of certain pigmented epithelial cells. The cells experimented with are situated in the organism between two entirely different elements, the retina and the choroid. Each cell is differentiated into two entirely different parts, one of which faces the retina and the other the choroid. But when the cells are cultivated in vitro, where the environment is uniform, the cell ceases to be different on opposite sides.

We shall now discuss those changes in the medium which affect not the form of the cells but their rate of growth. Plasma was originally used as a culture medium for the following reasons:

1. It furnished what seemed to be a natural fluid similar to that in which the cells are bathed in the organism.

2. On clotting, it furnished a framework for the cells.

3. It would, supposedly, furnish the necessary nutrition. It was soon found, as we have said, that the addition of embryonic tissue juice was necessary for indefinite growth in vitro. Apparently the cells were unable to get nourishment from the plasma. The proof that cells obtain their nourishment from the embryonic tissue juice rather than from the plasma was the fact that they grew just as well in a suitable synthetic salt solution plus embryonic tissue juice as in plasma plus embryonic juice. It was also found that with a constant quantity of embryonic juice, better growth was obtained with plasma from a young than from an old organism. This indicates that plasma from an old organism contains substances unfavorable to growth in vitro. It was definitely found that the older the animal the greater the inhibiting effect of its plasma. Carrel set out to prove conclusively that it was the actual presence of an inhibitory substance, and not the mere absence of a growth accelerating substance that made old plasma less favorable than young plasma. He did this very simply. If an inhibitory

substance is present, the greater the concentration of plasma in the medium the greater the inhibitory effect would be, while, if an accelerating substance is present, the greater the concentration of plasma, the greater the accelerating effect. Carrel found that the inhibiting effect increased with increase in concentration of plasma. Furthermore, in plasma from an old animal where the inhibitory substance is present in large amounts, the increase in inhibitory effect increases rapidly with the increase in concentration of plasma, while in plasma from a young organism in which less of the inhibiting substance is present the inhibitory effect increases slowly with increasing concentration of plasma. All these experiments were carried out on Carrel's 10-year-old strain of fibroblasts. The rate of growth of these in certain media was definitely known. He was, therefore, able to realize an old hope—of being able to use a pure culture of fibroblasts as a reagent for detecting some of the modifications occurring in blood plasma under the influence of age. Applying these results to the organism, we see that tissues cease to grow in the adult, not because the tissues in themselves become old in completing their necessary life history, which involves old age and death, but that their powers of growth cease because of inhibiting substances present in their environment.

Carrel took fibroblasts from organisms of different ages, put them in identical environments, and found that although their initial rate of proliferation varied with the age of the animal from which the tissue was taken, after several days the rate of growth was the same for all the tissues. This proved that for a given type of tissue the rate of growth was the function solely of the environment. Hence, if the environment is properly adjusted, immortality of tissues is attainable. Carrel's chick fibroblasts have now been actually growing for ten years, and there is no reason to believe that they cannot continue to proliferate indefinitely. So far the fibroblasts have lived much longer outside of the organism than they probably would have inside the chicken. It is true that a few chickens are reported to have reached the age of thirty years—but they are very rare birds. It has been calculated that if all the tissue coming from the original culture of a few square mms. in area had been preserved and cultivated further, the total volume would now surpass that of the sun. This shows that potential immortality is not restricted to plant tissues, and to certain protozoa, but that it is also a property of the tissues of the most highly developed animals.

The medium, then, determines the rate of growth of fibroblasts. What substances can be added to the medium to stimulate growth? It was found out very early that embryonic tissue juice was by far the most effective of such stimulating substances. This was to be expected, since embryonic tissue is the most rapidly proliferating tissue in the organism, and the most hardy, longlived in vitro.

Our knowledge of the factors accelerating growth was greatly increased when Carrel managed to get a pure culture of large mononuclear leucocytes. When an extract of the medium in which the leucocytes were grown was added to the medium in which fibroblasts were growing, the rate of growth of the fibroblasts was increased. Thus, by using the fibroblasts as a reagent, to test the effects of unknown substances on growth, it was found that leucocytes in vitro secrete substances which have the same stimulating effect as embryonic juice. The next step was to demonstrate that leucocytes secrete similar substances in vivo. Carrel produced experimentally connective tissue inflammations and peritoneal exudates, which contained large numbers of leu-

cocytes. Extracts from the inflamed connective tissue and from the exudates were found to have the same stimulating effect on the growth of fibroblasts in vitro as have embryonic juice and extracts from the medium in which lucocytes have been cultivated in vitro. We thus arrive at the conception of the leucocyte as a cell retaining its embryonic character throughout the life of the organism, as a wandering unicellular gland secreting substances which stimulate growth. As we have said before, the presence of inhibitory substances in the blood, which increase in amount as the organism grows older, effectively prevents the proliferation of tissues in the adult organism. There is one conspicuous exception to this: In the regeneration of wounds in even extreme old age, we have a proliferation of tissue recalling the rapid growth of embryonic tissue. It is striking that this rapid growth in an old organism in the regeneration after a wound is accompanied by the presence of leucocytes. Apparently, in wounds the leucocytes are active, not only in opposing bacterial invasion but also in secreting in the tissues the material necessary for cell multiplication. Furthermore, it is well known that if the wound is not irritated, healing is greatly delayed. It is also known that irritation causes the invasion of leucocytes. We can now appreciate the true significance of irritation, and of the leucocytic invasion of wounds. The conception of the leucocyte as a protective mechanism against foreign bodies must also be extended. The leucocyte in vitro as well as in vivo has powerful phagocytic powers. But in addition leucocytes have the property in vitro of responding to a foreign protein, such as casein, by the increased secretion of growth-promoting substances. This reaction of the leucocytes is immediate. Fibroblasts respond in vitro to an antigen, such as a foreign protein by the production of antibody, but this immunization of the fibroblasts begins only after four days. This would indicate that the first reaction to a foreign protein is not the production of antibodies by the tissue cells but the secretion of growth-promoting substances by the mobile, unicellular glands, the leucocytes, which wander to the spot of infection.

This new knowledge of the functions of leucocytes, as well as the discovery of the changes in the blood with age, was made possible by the in vitro technique. When we consider that most of the striking results were obtained within the past year or so, there is every reason to believe that this method will yield many valuable results in the near future. The work on tissue cultures has just begun.

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INTRAVITAL STAINING AND ULTRA-VIOLET PHOTOGRAPHY

IN considering this subject, it is deemed advisable to preface a few generalities concerning its importance to the field of anatomy as a whole, and specifically to that of histological research. The primary service of anatomy to medicine is contained in the fact that it enables the scientist to understand and then to correlate the mechanism which carries out the functions of the living organism, and it is to the understanding of this mechanism in its finer and more fundamental details that histology contributes for the most part. Now histological study for a long time consisted solely in the microscopic observation of animal structures which had been prepared from tissues after death, and subjected to fixation and staining processes; these factors were considered by many to have so acted on the delicate cell components as to render all observations invalid more or less according to the circumstances involved; it was argued that post-morten autolysis, destructive action of dyes, and the like, had produced afterfacts which jeopardized the very foundations of histological belief. In response to these accusation have arisen the investigations of cell and tissue structure through intravital staining and ultra violet photography. Here the structures are studied in the living state, or under conditions approximating it sufficiently closely, so that it is possible to verify, and in some cases improve on, the theories and conclusions of previous microscopic investigations.

In the consideration of intra-vital staining, which will first be dealt with, it is primarily necessary to bear in mind that it is a method, and not a theory, so there must be a lack of continuity between parts of the work done by different individuals; again, the subject, though still comparatively new, has grown to proportions which prohibit detailed examination in a paper necessarily so short; it will therefore be deemed appropriate to restrict this exposition to more general treatment, with specific presentation of only a few important or typical examples, leaving it to the reader to satisfy his further curiosity from the appended bibliography.

The history of intravital staining dates from the last quarter of the past century, and its inception is associated with, if not ascribed to, the work of Erlich, who stood out as the pioneer, and upon whose leadership subsequent investigations have largely depended. It was found that certain dyes, such as methylene blue, neutral red, or trypan blue, could be injected into the living organism, and that the tissues, though unharmed physiologically, would receive the stain, and could be examined microscopically, either by taking a section from a freshly frozen piece of living tissue, or by actual examination of the organism itself while yet alive. The technique is exceedingly complex and difficult, requiring extremely careful manipulation and exact conditions in order to preserve as nearly as may be the normal state of the living material.

Probably the most exhaustive and brilliant collation, as well as investigation of intra-vital staining, has been done by W. V. Möllendorf, whose article in the Ergebnisse der Physiologie, Vol. VIII (1920), gives an account of various applications of the methods, together with results obtained. First of all it was found that a typical cell on being subjected to vital stain, would appear to absorb it in granular deposits within the cytoplasm. These might later merge in a definite form, or collect in a group, and be extruded with waste or unused material. Now it was found, furthermore, that certain cells, or groups of cells, would seem to exercise a selective sort of function, so that while one set might take a certain dye another would not. Also, as might have been expected, different dyes affected the same cell in strikingly different ways, and one type of stain had varied effects on cells of unidentical character.

There are several methods of treatment; the dye may be injected intravenously or by peritoneal injection, or it may be applied to freshly excised tissue from a living animal. Subcutaneous injection may be used for some types of work, but its effect frequently coincides with that of the other methods.

Möllendorf describes the successful staining of many types of tissue and organs, eg.: Central nervous system, endocrin glands, lymph glands, genito-urinary organs, blood, muscle tissue, mammary glands and others. In the demonstration of nerve-endings, the use of vital staining is very valuable, and is regarded by some authorities as superior to other methods. In some instances it is quite certain that the value of vital staining has transcended all other procedures for investigation of cell structure; an example of this will be given at a later point.

Authorities differ very strikingly on the precise nature of the staining action—some aver that the dye enters into chemical relationship with the protoplasm of the cell itself; others claim that unless the stain is of a destructive, or at least reformative nature, it merely enters the cell body with the ordinary food of the cell, and remains suspended like other unused material in the cytoplasm until execreted. This is closely linked with the belief of yet another "school" which holds the cell action to be a sort of phagocytic mechanism, simply seeking to defend the organism against foreign material. This is supported by the observable actions of leucocytes on vital stain injections, as well as by other factors, but the question as a whole is handled in greater detail by another paper in the series on living cell reactions to foreign bodies.

Before going into the more detailed examples of intra-vital staining, it is proper to mention the physiological significance of this method. Obviously the value of such a process is very great, for by it the physiologist is enabled to observe the actual behavior of the living cell, noting details of movement and function which no series of preparations or pictures can quite duplicate. As an example of this we have the classic work of E. R. and E. L. Clark on the vitally stained tails of living tadpoles (c. f. Amer. Jour. Anat. XXI, Anat. Record XIV and XV) in which they observed cell and blood reactions over long periods through the conveniently transparent caudal appendages of their subjects.

One of the most significant pieces of work in the field of intra-vital staining was that done by Evans and Scott, whose report in Publication 273 of the Carnegie Institute "On the Differential Reaction to Vital Dyes Exhibited by the Two Great Groups of Connective Tissue Cells" stands out conspicuously

as an example of what intra-vitam dyes can do. These brilliant workers conducted a series of exhaustive experiments with different dyes under varying conditions, and their conclusions are here appended:

"The two great cell strains of mammalian connective tissue; the fibroblasts and macrophages—exhibit a pronounced and characteristic difference in their reaction to intra-vitam acid dyes," they point out. This is shown by the distinct responses to dyes, in the size and number of dye 'granules' and associated characterstics. Mitochondrial apparatus of these cells is apparently not stained electively by these vital acid dyes. The dyes do not enter into chemical combination with the cells, but are accumulations of high-colloidal, flocculated or crystalline dye-stuffs in fluid. The ingestion of the dyes is said to be associated with a process of segregation from the living cell protoplasm by an "apparatus" composed of granules, vesicles, and vacuoles created often by the cell in addition to those present. It was shown that the macrophage stored *more* dye, but less permanently than the fibroblast type of cell.

The physiological and histological importance of such observations is too obvious to bear elaboration; it but remains to point out the value of vital stains in another field, as shown by Bensley in his Harvey Lecture (1914-1915) on the "Structure and Relationships of the Islets of Langerhans." In this remarkable announcement, Bensley pointed out that he had been enabled to settle an old and long-contested point concerning the islets in the pancreas by a system of vital staining, in which he was able to demonstrate by two different stains the islets and the ducts of a single pancreas.

An injection of janus green (solution I in 15,000 normal salt) into the aorta promptly stained the whole pancreas blue; if the pancreas was now covered and air excluded, the tissue reduced the dye to a safranin, but the islets proceeded so slowly in this matter that presently they were the only blue part, the rest being intense carmine in color. The whole might now be fixed permanently by ammonium molybdate injection in the ducts, alcohol fixation, and toluol clearing. Then by breaking it up into fragments, and putting these on slides it was possible to count every island without sectioning, and without fear of recounting any one; also one could ascertain the proportion of islet content. Neutral red injections gave the same opportunity, but could not be made permanent. Now an injection of pyronin of methylene blue was found effective as a strain for the duct system.

By the complete survey which these invesigations afforded, Bensley was able to make his conclusions on the islets of Langerhans which have since proved so valuable to the field of medicine.

These are only isolated instances which are taken from the great mass of data to show that intra-vital staining, however new, has taken its place as an accomplice and assistant to histological study in particular, and science in general.

Ultra-Violet photography, like Vital Staining, is also a method, and one that in a general way pursues the same ends. While the chemist endeavors to form a philosophical and pictorial conception of life processes in terms of molecular arrangement and change, it has been the histologist's ambition to observe with his own eye these phenomena being carried out. Vital staining permits of this in some measure, but photography with ultra-violet rays opens an even greater field. Vital dyes show us at best a somewhat abnormal condition, but roentgen rays not only exhibit living matter but show it in the unstained state. It is difficult to overestimate the importance of this. The

cell which heretofore revealed its form only through the agency of rather obscure reactions—a word in itself implying change—may now be photographed in the mormal fluid medium with great precision. Furthermore, the method has a marked embryological value, since the subject may now be studied by a series of photographs of the same cell rather than by a number of stained sections taken from different cells. Aside from these considerations, which have an enormous philosophical importance in the field of biology as a whole, this method marks a tremendous advance in the purely mechanical problem of microscopic technique. This comes about through the employment of the short wave length, which, as will be explained below, constitutes the only known method for increasing the magnifying power of the present microscope. This was the twofold stimulus that urged Boys, Siedentopf, and Kohler to make their initial investigations of what had been up to that time an entirely untouched field.

It was Siedentopf, in 1903, who indicated the possibilities of visibility of ultra-microscopic particles for fractions of wave lengths of light, and in 1904 August Kohler announced the result of his researches, which have since become classic in the annals of microphotography. The technique which he developed, wherein he employed fused quartz lenses exclusively, following the earlier suggestion of Boys, remains, with a few slight modifications, the standard of today. It must be borne in mind that even with this precedent, ultra-violet photography is far from simple. The obstacles in the path of an investigator are very definite. To begin with, the apparatus is exceedingly complex and expensive. Furthermore, it appears to be enormously difficult to master the mere details of operation, and the success of a photograph is highly problematic. In fact, Ernst and Wolbach declare that a man who is proficient in ordinary microphotography requires several years of experience with ultra-violet work in order to obtain even a reasonable degree of success. However, the potentialities of the method seem great enough to compensate more than adequately for this expenditure of time.

Abbe, in his study of microscopy, came to the conclusion in 1876 that microscopic efficiency depended upon magnifying power, perfection of the union of rays, and the size of the aperture admitting cones of light. He further declared that in the first and last of these essentials efficiency had already been obtained, and to perfect the second one he developed his apochromatic microscope. Some time later he qualified his comments upon magnifying power by asserting that efficiency had been obtained only in respect to visible light, and that advances were possible in this direction by the use of light rays beyond the violet end of the visible spectrum. This conclusion rests upon the principle of resolution. With ordinary light wave length fluctuates within fairly narrow limits. For white light we may take the length of the central yellow green rays as standard, that is, about 550 rr. in air—with a slight variation for media of different refraction. By the use of filters, then, which exclude other lights, shorter wave lengths may be obtained which are more easily photographed than seen, although this process requires special apochromatic devices to correct the resulting observations. The decrease in the wave length brings a corresponding increase in the resolving powers of the lens, and this leads directly to greater possibilities of magnification, and greater clarity. The term "resolution" may be explained as follows: Let us assume an ideally perfect double convex lens, beneath which is a perfect point source of light—A. The rays will diverge until they reach the lens, and

then converge as the central part is retarded. They will meet at a point, giving an intense point image. It is obvious that this point will be surrounded by a diminishing series of dark and light hands caused by the coincidence and mutual neutralizing effects of the vibrations. Manifestly the image from another point source of light—B—will, with a certain degree of proximity, be merged with the image of A and but one light will be seen. This condition is known as failure of resolution. It follows that in order to obtain resolution the first dark hand from A must be superimposed on a similar one from B to secure separation of the images. That is to say, the distance between the points of light must be the equivalent of two one-halves of a wave length. This interval will induce the crests and troughs to coincide, and produce the essential dark intervening hand. The implication is, therefore, that any given ray from point B must travel just one wave length further than a corresponding one from A. The geometry of the figure thus constructed will show us that the shorter this wave length is the nearer A and B may be together and still retain their duality of image. This is called greater resolution, and on it obviously depends the possibility of increased magnification and clarity in microscopic work. Since ultra-violet rays have the shortest known wave length, their relation to microscopic efficiency is apparent.

The actual process of photographing these rays presents numerous technical difficulties, and the apparatus, originally designed by Kohler, has been somewhat modified by Barnard. The following description is taken from the last named author. The objectives are three in number, made of fused quartz, and possessing a focal length of 6 mm., 2.5 mm. and 1.7 mm., which correspond to an effective numerical aperture of 2.50, 1.7 and 0.7 respectively. The last two are glycerine immersion, the refractive index of glycerine being 1.447. The cover glasses used are of optically worked fused quartz, the slides also being of fused quartz; they are mounted on a special carrier insuring uniform distance from the objectives. The equipment includes five quartz occulars, having an initial magnification of 5 to 20, which gives the camera a magnification power of 200 to 3,600 diameters. Barnard has not yet been able to photograph clearly with the highest magnification. The substage condenser is of quartz with an adjustable duplex top. Light is furnished by a high tension discharge in air between cadmium or magnesium electrodes, these metals being chosen for the intrinsic properties of their ultra violet spectra. A spark gap of 5 cm. receives the heavy discharge of low potential from an induction coil of special design. This spark throws its beam through a quartz lens at right angles to the optic axis of the microscope, and is filtered by two quartz prisms of opposite rotation. The image of the spark is focused by means of a disk of unranium glass placed below the substage condenser. To focus the final images, a fluorescent searcher eye-piece is mounted above the occular, and the focusing is done by means of an auxiliary magnifier. After visible focusing, the fine adjustment is moved a predetermined amount for any wave length being used. The camera now replaces the searcher eye-piece, and the image is projected so as to be in focus for the plane of the plate. The latter must be of exceptionally fine grain, having the maximum quantity of silver salt and the minimum of gelatine. Such a plate is made by the Eastman Kodak Company. An exposure as short as 2 seconds may be successful, although it is commonly longer. This method of ultra-violet photography, in which great care is taken to employ monochromatic light spectroscopically analyzed, eliminates achromatism entirely.

Cells and tissues to be examined are mounted unfixed, undried, unhardened in such fluids as normal saline, lachrymal, or Ringer's solution. Inasmuch as death results in increased opacity to ultra-violet rays, it is essential to have the material in the living state. The physical principle involved in this photography is embodied in the fact that different substances have different absorption indices for ultra-violet rays, and when the picture shows differences in substance throughout a cell it is safe to infer that they correspond to structures in the cell. Bovie has conducted elaborate researches into this aspect of the subject, using fluorite rays and reducing his results to tabular form, which may be employed in the future to determine the chemical nature of the structures observed. A very suggestive field is thus offered. These considerations serve to show more clearly the tremendous importance of the method in its revelation of living phenomena. It may be argued that the rays themselves alter the structure of the cells, which would undoubtedly be true if the exposures were several hours in duration, but Bovie, in his experiments with paramecia, has shown that complete cytolysis results only after an exposure of far longer duration than that necessary for photography. By submitting these forms to the action of the light for relatively long periods, he discovered the only change to be an inhibition of cell division, which after a time was followed by a great acceleration, showing the compensatory tendency of living material.

In attempting to collect some results of this method which would be striking enough to justify its enormous theoretical value, one encounters considerable difficulty. Its amazing technical complexity, coupled with the paucity of investigators, makes choice of illustration quite limited. If the results have not as yet been the basis of new theories or the means of refuting old ones, it has at least corroborated existing conceptions. The pictures produced may not have revealed anything radically new or important, but they afford a thoroughly satisfying certainty that many of our present beliefs are true. These photographs have revealed living forms with a fineness and clarity of detail never achieved before in microphotography. We can derive much satisfaction from the knowledge that there is at our command a technique capable of establishing any obscure point in histology whose importance warrants such laborious investigation. In a paper of this nature where the reproduction of plates is impossible, it is obvious that a detailed description of a great number of photographs would be cumbersome and valueless. It may be of some profit, however, to indicate the general nature of some of them. Ernst and Wolbach have produced remarkably clear pictures of the striations in potato starch, together with some highly suggestive stages in the cytoplasmic reorganization of bacteria. The typical ultra-violet plate is devoid of high lights and shadows owing to its thinness, but lines of demarcation are extraordinarily distinct. Kohler's initial results in the field are also well worthy of mention. At a magnification of 1,300 diameters he obtained a photograph of an epithelial cell from the gill plate of Salamandra maculosa which is in the process of mitotic division. It is a late prophase and the amphiaster is very distinct; the symmetry of this figure, together with the decisive etching of the astral rays is most interesting. In two photographs of living filaments of Cyanophycea he obtained an almost complete series of mitotic figures. All Kohler's work is striking, but essentially he was a pioneer, more concerned with the physical problems of his art than with the biological implications thereof. Research in this field is now being actively

carried on, notably by S. E. Barnard, and by Bovie and Mudd of Harvard. The last-named investigator expects shortly to add to the literature of the subject, and to announce the result of several years' work at Cambridge.

The value of the methods which we have discussed lies ultimately in the possibilities which they admit rather than in what has been accomplished hitherto. But anything which serves to lessen the gap between scientific conceptions and the almost infinitely diverse complex of unseen form and movement upon which they rest demands the consideration of all those engaged in the pursuit of fact. It is impossible to foretell the effect of these two new departures in the future on such an established science as histology, but we venture to predict that should any discovery radical enough to change the whole tenor of the field be made, it will come from one of these sources.

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THE INTERSTITIAL GLAND OF THE TESTIS: ITS HISTOLOGY AND SIGNIFICANCE

THE interstitial cells of the testis were first reported and described in 1850 by Leydig—and consequently have been termed the Cells of Leydig. He described the cells as round and clear, analogous to embryonic connective tissue, occasionally with vacuoles, and always containing fat and pigment granules. Four years later Kölliker showed their presence in the Mediastinum and connective tissue septa, and also under the Tunica Albuginea, as well as between the seminiferous tubules, as related by Leydig. In 1903 Bouin and Ancel in view of the probable glandular function of these cells, applied to them the appellation interstitial gland of the testis.

Intestinal cells exist in all the vertebrates that have been examined, with the possible exception of a few birds. The degree of development of the cells is subject to extensive variation—nothing like uniformity being discernible in any two species.

According to Bouin, Ancel, Chapin and others these cells make their appearance in the genital tract and become functional before the formation of definite germ cells. Whitehead, working with embryonal pigs demonstrated that the intertubular tissue of the testis in stages immediately preceding the appearance of interstitial cells is a "Mesenchymal structure derived from the mesenthelium of the genital ridge." Histologically this is a connective tissue syncytium consisting of cells and an exoplastic network of fibrils. The cells are practically devoid of cytoplasm and consist of naked nuclei with fragments of cytoplasm at one pole. The interstitial cells develop from this tissue by growth of cytoplasm. The early cells are quite plainly branched. These branches, however, disappear in subsequent rapid growth of the cells in size and numbers.

In the pig these cells pass through two phases of growth with an intervening phase of atrophy. The cells appear at the 2.4 cm. stage of the embryo, and grow rapidly to the 3.5 cm. stage, then follows the period of atrophy during which the cells return almost to their first stage of bare nuclei. The climax of this process is reached in the embryo of 14 cm. Simultaneously there is an extensive growth of the seminal tubules so that they are greatly convoluted and the intertubular spaces correspondingly narrowed.

The second stage of growth of these cells begins at the 20 cm. stage and attains its maximum in the embryo at 28 cm. very near to term—at which time they have increased enormously.

In the human embryo, Felix found that the interstitial cells develop early, i. e., in embryos of 45 mm. before the formation of definite germ cells. The early cells are easily recognized by their large size and nuclei which contain a dearth of chromatin. After the fifth month there ensues an apparent quiesence, during which formation ceases until after puberty, when they

appear again in great numbers. Another phase of retrogression follows, which in turn is followed by a renewed increase.

In the adult human testis interstitial cells vary considerably in numbers as between individuals. No marked difference between right and left testicle has been noted. They occur singly, in groups or in acinus-like clusters, and are closely related to blood vessels. They are easily identified and can be distinguished from connective tissue cells by their general appearance, and by the fact that they do not stain with vital dyes such as trypsan blue. The nuclei are usually single and spherical, but double nuclei have been seen. The nuclei are poor in chromatin and rarely contain distinct nucleoli. In lower forms, and probably in man, the cytoplasm of these cells has a striking affinity for mitochondrial stains. This may be due to a slightly acid reaction on the part of the cytoplasm. Winiwarter found in man that mitochondria, occurring as granules and rods with an occasional filament, were fairly evenly distributed throughout the cytoplasm. Small clusters are exceptional. The same investigator described the centrosome in the human interstitial as spheroidal or rod like. If the nuclear membrane is indented, as is often the case, the centrosome may be found in the concavity . Two centrosomes may occur in the singly nucleated cells, and in the bi-nucleated as many as four have been seen. No radial arrangement of cytoplasm granules exists about the centrosomes to indicate it to be a dynamic center.

Fat granules appear in various amounts. They occur in clumps and are distributed through the cytoplasm without apparent order. They are believed to increase with age.

Pigment granules appear at about 21 years and become abundant. Lehrt believes this pigment to be a lipochrome. Often there is a sharp contrast between the pigmented and non-pigmented portions in the same testicle. This has led to the belief that the pigmented cells are undergoing degeneration, although there exists no noticable difference in the size of the cells or in the nucleus—cytoplasmic ratio.

Duesberg alone has studied the reticular apparatus of these cells. No trace of such a system is visible in living unstained cells, but by Cajal's uranium nitrate method a network was shown. It varied somewhat in different specimens, but evidently indicates some regional variation in the properties of the cytoplasm because the location of the network is quite definite—always in close assocation with the centrosome.

In discussing the significance of the interstitial gland, we shall first try to prove that the testis, specifically, the interstitial cells therein, may be called an internal secretory organ. Next, in describing the functions of the internal secretion we shall deal with the influence of the testicular hormone upon the development of the generative organs and upon the nervous and particularly the vasomotor apparatus. Following that, we shall attempt to explain the phenomenon of rejuvenescence by testicular transplants and its relation to the interstitial gland. We then will discuss the relationship of the latter to other endocrine glands, its influence on metabolism, and finally, the question of sex intergrades.

Significance

We found that in the primitive organisms there was no specific tissue set apart for reproductive purposes. With the evolution of greater complexity of structure, there was set apart a definite reproductive tissue. This tissue is

differentiated early in the embryonal life of the individual organism. Associated with the division of the plasms, the germ plasm from the somatoplasm, there appeared reproductive tissues of two types; that giving rise to male and that giving rise to female elements. The appearance of these two types of reproductive tissue assocated with somatic divergences, established sex.

Some somatic differences consisted primarily in the system of outlets, the genital organs so adapted as to insure the more certain fertilization of the egg. There arose other characteristics associated with this phenomenon, those by which the sexes are differentiated, namely, the secondary sexual characteristics.

In addition to the structual and functional differences between the sexes have arisen the sexual instincts. In the higher forms these instincts or as many prefer to call them "reaction impulses" bring the male and female together at the breeding seasons, control the behavior of the individuals towards each other—courtship, the union of the sexes—copulation—or its equivalent, and finally direct the various activities involved in the building of the nest and the rearing of the young.

There is no doubt that the secondary sexual characters in the male are due to an internal secretion on the part of the testes. It is well known that castration in man before the age of puberty prevents the growth of hair on the face and arrests the growth of the thorax, pelvis and the larynx. Thus it is possible to preserve the voice of childhood. It is stated that the practice of castrating boys for the cathedral choirs in Rome was carried out until 1878. Male sopranos or sopranoists were formerly a leading feature of musical life. Perhaps the most convincing evidence of the dependence of the somatic structures related to sex upon the primary reproductive organs is afforded by the results of cross grafting of gonadal tissue into previously castrated animals. Steinach and Sand have clearly shown that the ovaries of the female rat and guinea pig can be successfully grafted into previously castrated males. The "feminized males" develop characters peculiar to the female.

The older physiologists exemplified by Nussbaum thought that the relationship between the interstitial cells of the testes and the secondary sex characters was accomplished by influences exerted through the nervous system. However, the results obtained by the gonadal tissue transplantation, as mentioned above, proves this theory untenable. It is interesting to note that preparations and extracts of reproductive tissue serve a similar purpose. (Massaglia.) Hence the testes may be considered as organs of internal secretion. The exact manner in which the gonads exert their influences upon the somatic structures, however, remains to be demonstrated.

At present there is no evidence that the secondary sex characters are in any way assocated with the activities of the sperm-producing cells. On the contrary there is a wealth of evidence which indicates that these characters are dependent upon certain cells lying in the interstices between the seminiferous tubules, viz.: the interstitial cells.

Many facts, demonstrating that the interstitial cells are responsible for the internal secretion of the testes, are revealed by the studies of the selective action of the X-rays, vasectomy and undescended testicles. The work of Ragaud and Subrenil shows that the action of X-rays upon the male testes renders the animal sterile while the secondary sexual characteristics are un-

impaired. Histological examinations have clearly shown that the X-rays while destroying the germ cells, fail to alter the interstitial cells.

According to Bouin and Ancel ligation of the vas deferens in animals results in the cessation of spermatogenesis. On the contrary, the interstitial cells retain their morphological and functional integrity indefinitely, and as long as these remain, alteration of the stigmata (general make up) of the sex does not occur.

Hyperplasia and hypertrophy of the interstitial cells appear always to accompany both imperfect development and secondary atrophy of the seminiferous tubules (Tuck). Cryptorchid animals or those possessing imperfectly descended testicles may be considered intermediate between castrated and normal individuals in regard to genital glands, sexual instincts and characters. Not infrequently there is a minimal development, or an entire lack of interstitial cells; consequently the apparent degree of maleness is determined by the degree of development of these cells. (Bouin, Ancel, Toolen.)

The question arises whether or not the sustentacular cells are partially responsible for the production of the testicular hormone. From the experiments of Hanes, it has been fairly definitely established that their functions are limited solely to the nutrition of the spermatic elements. On the other hand the morphological position of the interstitial cells, as seen from their intimate relationship with the rich capillary meshwork surrounding them, suggests the possibility of a close relationship with the body fluids or distributors of internal secretions.

The facts that the accessory sexual organs and the secondary characters of the sex appear, and are maintained in association with the interstitial cells is alone strong evidence in support of the theory that these cells are responsible for the hormone production of the testes.

We shall now discuss the functions of the internal secretions of the testes. We find that in the development of the embryo vascularization of the generative tissue precedes the appearance of sexual genitalia. Therefore, it would seem that some secretion passing from this tissue into the blood influences the appearances of these organs. In the case of two sexed twins of cattle the female as a rule is sterile, whereas the male is normal and fertile. The female, commonly called the free-martin, has the internal genital organ of a male and the external genital organs of a female. The facts that the chorionic blood supply of the twins is common, that this chorionic fusion occurs at or about the time of sex differentiation, and that the testes of the male begin to develop before the ovary of the female, point to the conclusion that some internal secretion from the testes passes into the common blood stream and affects the sexual developments of the female. Such an explanation of the factors, resulting in the formation of the free-martin, lends weight to the argument of Van der Broek, who contends that the cause of pseudohermaphroditism is an imbalance of gonadal hormones between mother and foetus. Puberty, a period ushered in by an enormous proliferation and activity of the interstitial cells, and ripening of the germ cells, is further evidence to show the influence of the testicular hormone upon the development of the generative organs.

What influence has the internal secretion of the testes upon the nervous structures and their functions? At puberty inactive and dormant nerve centres acquire specific sensibility. This *storm and stress* period and the psychic unrest accompanying it, is paralleled by an increased vitality and great nervous

activity in most animals during breeding season. Removal of the testes of the frog results in the loss of these nervous characteristics of the rutting season. Testicular transplants in such frogs are sufficient to establish these seasonal changes; hence, the testicular substance either acts directly upon the nerves or upon the muscles supplied by certain of them.

It is generally accepted that stimuli acting upon our nervous system control our behavior and temperament. The dispositional characteristics of the *eunuch* show an influence of the internal secretion of the testes upon the nervous system, similar to that of external environmental stimuli. It is reported that eunuchs of Constantinople are avaricious, illogical, obstinate, possess little judgment and accept information without proof. Their mentality is often deficient and they are very fanatical.

From experimental work of Wheelon and Shipley it is found that the presence of testicular transplants in dogs previously castrated is sufficient partially to reinstate normal vasomotor irritability. This shows that castration results in a lowered irritabilty of the vasomotor apparatus because of interstitial want.

Recent experiments by Sand and More have shown that testicular transplantations have resulted in a phenomenon commonly called rejuvenescence. The "puberty gland" of Steinach, as mentioned before, is meant to designate the internal secretory organ of the testis—or the interstitial gland, in differentiation from the external secretary organ of the testicle. In a recent article in the New York Medical Journal, Dr. C. K. Lydston describes a remarkable case of testicle implantation from its clinical aspect, it is as follows:

Age of subject—34 years; suffering from hypopituitarism. Subject was badly nourished and psychically much depressed. The development of sexual organs was about that of a child one year of age. No sexual activity ever had been experienced. Patient was inefficient mentally, physically, and sexually.

It was an apparently hopeless case, his age seeming to be an unsurmountable obstacle to success. Finally, a left scrotal implantation of a single testicle was performed upon this patient. The material used was taken from a subject 18 years old, dead of skull fracture. The gland was removed 24 hours after the death of the donor, and refrigerated for 30 hours. Primary union took place, and the patient was up and about with suspensory on the 10th day.

Considering the age and other circumstances the effects of implantation in this case were astonishing. Marked improvement in nutrition and general health of the patient was evident within a few weeks. His color improved, and he became cheerful. There was an increase of eight pounds in weight. Within eight weeks after implantation the patient complained of frequent and violent erections, and some days later indulged in coitus, experiencing sexual manifestations at various times thereafter.

Since the operation there has been a marked increase in the growth of the axillary and public hair, and the patient now is compelled to shave weekly, an entirely new experience for him. His mental and physical efficiency have greatly increased after the operation. The testes have greatly increased in size as is true of the penis. Orgasm and emission both are experienced. Conditions still are improving.

In the author's opinion, the result in this case will be permanent, because of the marked development of the patient's own testes. It is believed that practically all, if not all, such subjects, if operated on, at or about the usual age of puberty, can be taken through puberty, and will show sex development,

with corresponding secondary masculine sex characteristics, approximating the normal more or less closely.

As to the permanency of cases like the foregoing, it is held that, even where, after a greater or less interval, a repetition of the implantation is demanded, the operation is still a great step in advance in the treatment of a class of cases hitherto regarded as hopeless. There is no question as to the uniform success of a properly performed implantation in such cases when performed at a reasonably early period after the loss of the patient's own glands. One is convinced that by implantations performed at varying periods the results can be sustained indefinitely.

In such a homo transplant as described above, there is an immediate loss of spermatogenic function with complete degeneration of the spermatozoa forming elements. The interstitial cells of the transplant remain and increase in number, retaining their staining properties the same as before the operation. Just how long the interstitial cells live and function after a successful homo transplant is still a question subject to lispute. It is believed that if there is a successful take, they will live indefinitely. If one does not have a take, the interstitial cells degenerate within a few months. In auto transplants the same phenomena occur as in the homo transplants. The only difference is that there is a larger percentage of takes.

All symptoms due to senility including sexual impotence, as a rule, improve after the operation. And this is what we must emphasize—the success or failure of the operation depends not only upon the ability of the puberty gland to renew or increase activity, but also upon the response that the other ductless glands, on the whole endocrinological system of the patient, will give to the renewed output of gonadal hormone. As long as the new activity of the interstitial gland lasts and as long as the other endocrine glands respond to its impulses, the patient's senility is postponed, partly at least, or its advance checked.

Whether a prolongation of life is obtained is impossible to say. So far, in no one of the successful cases has the patient come to a second senility or has died, except, of course, of intercurrent diseases, like pneumonia. But one thing can be said already as based on experience. Man's life of activity, that part of man's life where he works and creates, can be prolonged in many instances. This, it seems is the important fact. A warning should be sounded against too great an enthusiasm and against raising too many hopes. Steinach's own words should be remembered, "That within modest limits the process of becoming senile can be influenced." It will be a matter of future study to determine its exact value to the individual and to society in all its economic and social consequences.

Relationship to Other Endocrine Glands

An intimate relationship exists between many of the sexual secretions and the hormones elaborated by some of the other ductless glands. As an example, the adrenals have an intimate relation with the ovaries and testes. Lesion of the suprarenal cortex, leading to increased secretions, turns the secondary characteristics of a female in any given case into those of a male and suppresses the functions of the ovaries. It is only logical to suppose that the suprarenal cortex when normal had been feminine rather than masculine in type (Bell). Abundant evidence shows that a large number of internal secretions are concerned in the development of the sexual glands, for sexual pre-

cocity can be associated with tumors of the adrenals, as above, and with disturbances in the hormonic balance (Cobb). It is a fair assumption, therefore, that there may exist gonadal stimulating properties in some of the other endocrine glands.

Influence on Metabolism

The influence of the internal secretion of the testes is to be noted in connection with the growth and general metabolism of the individual. At puberty and during the breeding season in the lower animals, there is a marked increase in the vitality and the metabolic activity of the animal. We have an increase of vigor both physical and psychical. An increase of the interstitial cells occurs. On the other hand diminution of the functions of the interstitial gland, for any cause whatever, is accompanied by senile changes very similar to those resulting from castration. From this we may infer that the interstitial hormone directly influences the increased metabolic rate common at puberty.

E. Allen has shown that the interstitial tissue of albino rats fed on a diet deficient in water soluble vitamines, is hypertrophied. A concomitant result is that these animals become sterile. This condition is analogous to the results following exposure of the testes to X-rays.

Normal growth and development of osseous structures is intimately associated with the elaboration of an internal secretion by the testes. Following castration endochondral ossification is stimulated and prolonged. The skull is altered with the result that the cranial capacity develops less completely than is normal. The expansion of the antrum of Highmore is retarded, as the result of which the face remains narrow. Steinach's experiments showed that "feminized' rats possessed a female form of pelvis. The reverse is true of "masculinized" animals. The fact that gonadectomy is frequently followed by an increased deposition of fat shows that there must be an intimate relation between fat metabolism and the testes. Such a deposition is also true in men and women of advanced age after the supression of sexual activities.

Sex-Intergrades

The phenomenon of sex intergrades contradicts the older conception that sex is an absolute attribute. Sex intergrades may be established experimentally in animals by the simultaneous implantation of gonadal tissue into previously castrated animals. These experiments were performed by Sand, Steinach and Boruttan. If the reproductive tissue of the two sexes were simultaneously implanted in a neutral animal, the interstitial tissue of both sexes intermingled in their growth and produced hormones. This resulted in the development of male secondary sex characteristics, the skeletal and muscular growth also being that of a male. Psychically, the individual behaves at times as a male, and at times as a female. As a male it will fight off other males, and seek and attempt to mate with rutting females. At cyclic intervals it behaves as a female and will be sought and courted by males. If, however, male interstitial tissue predominates, male characteristics will predominate, and vice versa. According to Biedl, it appears as though the gonadal hormones are not antagonistic, but exert their influence in proportion to the degree and quantity of their delivery to the somatoplasm. Goldschmidt contends that no animal including the human is either purely male or female, but that each has the potentialities of both. In the light of modern experimental proof, hermaphrodites and pseudohermaphrodites, and many other persons considered

abnormal, should be considered as intergrades. These cases, therefore, are not pathological, but simply natural variations, the result of the chromosome complex, and of abnormal internal secretion of the gonads. Normal sex must be considered as the result of complete differentiation of the sex gland anlage into one or the other type of interstitial cells.

Based upon the evidence we have collected we believe that the following conclusions may be safely drawn. It appears certain that the internal secretion of the testis definitely influences somatic growth and stimulates the development of the secondary sex characteristics.

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THE REACTION OF LIVING CELLS TO FOREIGN BODIES

THE reaction of living cells to foreign bodies involves practically the whole of medicine. Naturally, in less than half an hour, we can consider only a small phase of the question. We shall not touch upon macroscopic foreign bodies such as splinters, bullets and safety-pins. Nor shall we consider food in the digestive tract. This paper will have to be limited to the finer reaction that takes place when certain microscopic substances and their toxins attack the living cell. Bacteria, however, are not going to be considered. Instead, we are going to give a survey of some recent experiments on the injection of different substances such as proteins, fats, erythrocytes, and various oils into living animals. The animal body is, as we have all learned, an accumulation of myriads of cells all working together. During years of evolution, these entities have developed a very effective protective mechanism. Various phases of this mechanism we are just beginning to understand. In the normal individual, everything is in equilibrium. When extraneous matter is introduced this equilibrium is upset. To restore it the body may form antibodies which neutralize the toxic effect of the foreign substance, or the body may send its phagocytes to ingest the foreign substance.

The study of the reactions of living cells to foreign bodies arouses interest in the relation of these cells to antigens and antibodies as contrasted with their reaction to non-antigens. It is, therefore, the purpose of this article firstly—to briefly review the nature of antigens, secondly—to present a series of experiments which tend to show their influence on antibody formation, and finally to observe the reactions of living cells to various diverse types of nonantigenetic substances.

When we look at the question from the chemical viewpoint, we note that there appears to be an essential difference between the reactions which are induced by simple chemical compounds and those which are incited by more complex substances of a protein nature. This latter group of substances incite reactions which are more or less specific in nature and which increase the resistance of the body against the substance that has induced the reaction. On the other hand, the non-proteins are involved in reactions which are not specific and which do not increase the defensive powers of the body so as to give it an immunity against the poison. The substances of the first group are non-antigenetic, while those of the second (more complex) group are antigenetic. By antigens we mean substances that are capable of causing the formation of specific antibodies which will react with the antigens. In general only proteins will produce antigens, although there are cases on record of other substances doing this. Not all proteins, however, will induce the formation of antigens. The derived proteins are deficient in this respect. Solubility of the protein seems to be necessary, as proteins which have been coagulated by heat lose their antigenetic properties. Non-coaguable proteins even after boiling retain their antigenetic nature.

It was formerly believed that the introduction of a protein into the body caused the formation of enzymes which destroyed and removed the foreign protein and that the excess of enzymes produced were the antibodies that gave an immunity. This theory has been accepted in part but with this modification: that it is the destruction of the foreign material which causes the antibodies to be produced in excess. In support of this view we have a number of investigations which show that the more readily a protein is broken down by proteolytic enzymes, the more easily will antibodies be formed. Gelatin is, however, an important exception to this rule, for although it may readily be disintegrated by enzymes, it fails to react as an antigen. In establishing this general theory the work of Ten Broeck has been most striking. He has proved that racemized protein, which in every way resembles simple protein save that it lacks optical activity and resists ordinary digestion, is non-antigentic in nature. Upon treating a sensitized guinea pig with racemized egg albumin no symptoms of anaphylaxis were noted. Treating a control animal with the same dose of ordinary egg albumin death was produced within two hours.

Attempts have been made for a long time to arrive at the composition of the antigens and antibodies. They were formerly considered of a protein nature. Recent workers who have obtained them in a greater state of purity report that they fail to respond to the protein tests. Warden since 1915 has spent much time on this subject. He has attempted to show that the specific antigens are fatty complexes which are peculiar to a particular protein and which serve to give it a definite chemical character. Thus its specificity depends upon the composition of the fatty acids which make it up and on the kind of surface which receives it. In this way we may conceive of an infinite number of antigens. Perhaps the most convincing of his experiments has been that on the antigen of anthrax. From cultures of this bacillus he was able to obtain sufficient fat to make an analysis. He discovered it contained caprylic, caproic, palmitic and oleic acids. About a half of the mixture appeared to be oleic acid. By trial mixtures, he found that one which contained 10% caprylic, 25% caproic, 5% palmite and 60% oleic gave immunity reactions which most closely approached those which resulted from the bacillus itself. Upon injecting protective amounts of this synthetic antigent into rabbits he has been able to make them withstand ten times the lethal dose of the bacteria. He has failed, however, with a hundredfold dose. As he did not need any protein from the anthrax bacillus to establish this immunity he concludes that aside from emulsifying power protein has little function in immuni y reactions. He accounts for his difficulties in preparing very powerful artificial antigens as the result of his inability to determine the exact composition of the antigen and the best method of dispersing it.

Many interesting experiments have been carried out in an attempt to discover the relation of these antigens and antibodies to the tissues. One of the latest theories is to give most of the credit for destroying antigens and forming antibodies to that mysterious organ—the spleen. The experiments to be described here are the ones in which foreign blood cells were used as the antigens.

In 1911 Luckhardt and Becht carried out an experiment on dogs from which they reached two main conclusions: 1, that the spleen fixes antigen, and 2, that the spleen is concerned directly or indirectly in the production of immune bodies (antibodies). These authors assumed that foreign erythro-

cytes are hemolyzed in the blood of the animal injected and that the spleen cells help remove them. They reasoned thus—one of the functions of the spleen is to destroy the worn-out red blood corpuscles of an organism. Why, therefore, would it not destroy foreign corpuscles injected into the blood stream? Since these injected corpuscles would be foreign to the organism, the spleen would react against these corpuscular antigens by producing great numbers of specific destructive agents (the antibodies in question).

Their method was to inject an optimum dose of goat's or rat's blood corpuscles (the antigen) into a dog's veins. When they removed the spleen of this dog, emulsified it and introduced it into the peritoneal cavity of a normal dog, they found the specific immune bodies in the serum of the latter. Evidently the spleen of the injected dog had formed excess immune bodies. The introduction of normal spleen into the peritoneal cavity is not followed by an increase of antibodies in the serum of the recipient. The introduction of any other tissues from the injected animal, whether heart, liver, bone marrow or lymph glands, did not give positive results. That is, only injections of the spleen of the injected dog could immunize a normal dog to the substance injected.

To verify, they also performed experiments on dogs without spleens and compared their reactions to the reactions of the normal dogs. The asplenic dogs did not produce immune bodies as rapidly or in so high a concentration as the control dogs.

What cells in the spleen, the fixed or wandering cells, possess this power?

In 1915 Cary, working on rabbits, claimed to have shown that the destruction of an animal's own red blood corpuscles is accomplished by specialized fixed tissue phagocytes (the endothelial and reticular cells) in liver and spleen. Cary called these fixed phagocytes hemophages. He further concluded that this hemophage activity increases directly with the number of erythrocytes injected and with the length of time these foreign corpuscles are allowed to stay in the rabbit's blood before the animal is killed.

Cary used washed beef corpuscles as the antigen and observed their effects in the blood stream of the rabbit. Normally there are not any active hemophages in the liver; the hemophages in the spleen are sufficient to destroy the erythrocytes. The ingestion of red blood cells by hemophages was indicated by a positive iron test showing that hemoglobin was present. Cary injected foreign corpuscles to see whether they or their products would also be ingested by the hemophages. As he expected, the hemophages of the spleen and also of the liver attacked the foreign corpuscles. Because liver and spleen endothelial and recticular cells gave a prussian blue positive iron test after foreign red blood cells had been injected, Cary concluded that the fixed cells could destroy antigens.

Cary, in concluding, went a step further than Luckhardt and Becht. Working on the known principle that foreign red blood cells introduced into an animal stimulate the production of specific antibodies, he proposed to show in a later article that the very cells which destroy erythrocytes help form their antibodies. Unfortunately, he has not as yet published such an article. But the work of Luckhardt and Becht and the work of Williams tend toward such a conclusion.

Williams, in his report, 1919-20, claims that the fixed tissue cells should

not get all the credit for destroying antigens, because the same cell types are not the active phagocytic agents in all cases. As he phrases it, "In some conditions, it is found that fixed cells, such as reticular and endothelial cells, are most active, while in other conditions, free cells, such as splenocytes, are the most predominant phagocytic cell type. These findings are to be correlated with the well recognized fact that the phagocytic cells have a selective action, some types reacting to one stimulus, some to another." Williams, is, of course, referring to the large, free-swimming mononuclears, which he terms splenocytes, normally found in the spleen. Though these may ultimately be derived from the endothelial cells (P. & S. Students' Report, 1921), Williams' technique (better than Cary's) indicates that where cells and cell fragments, erythrocytes in particular, are concerned, the splenocytes (free cells) are the first to act and they continue to act as the main phagocytic agents.

The stimulus employed in his experiment was washed pigeon corpuscles injected into the circulation of the rabbit. He found that rapid destruction (hemolysis) of the foreign corpuscles ensued and, as an almost immediate effect, was followed by the release of mature and immature blood cells (including polymorphonuclears and myelocytes) from the bone marrow. Both the hemolyzing blood of the pigeon and the bone marrow cells of the rabbit, being brought by the circulating blood to the spleen and then delayed within the cavernous blood channels of the pulp, were thus exposed to the phagocytic action of the splenic cells.

Williams interprets the "bone marrow crisis" as he terms it, to be the result of the toxic material set free in the hemolysis of the pigeon blood. In the spleen he found that the bone marrow cells were ingested by the splenocytes which grew larger with their contents and showed, in some instances, as many as twenty cells in one focal plane. Towards the end of digestion of their inclusions the splenocytes returned to normal size. He also found that the endothelial cells as such were little concerned in phagocytic activity in this experiment, as determined by the iron test, miscroscopic examination, etc.

Keeping the results of these experiments in mind, it is possible to reach the general suggestive, if not positive conclusion with Cary, that the cells which destroy erythrocytes participate in the formation of their antibodies, especially when we consider the possible significance of the bone marrow crisis in Williams' experiment.

Independent of but coördinated with this method of attack is the more general method explained on physico-chemical grounds, surface tension, etc., of the invader attracting hosts of protecting bodies, especially leucocytes. As you know, phogocytosis is not concerned alone with destroying dead cells, but may go on in any tissue. Now we are going to look at phogocytosis as a general fight against foreign stimuli.

A new method of aproach to this problem has been suggested by Eleanor and Eliot Clark. For 12 years these experimenters have been studying the reaction of connective tissue cells, wandering cells, and lymphatic and vascular endothelium in the tadpole's tail towards various injected substances. These Clark experiments are very important for they furnish us with accurate records (drawings were made with the aid of a camera lucida) of the reactions of living cells in the transparent fins of the tadpole's tail. The Clarks chose normal larvae, anaesthetized them in chloretone solution, with fine

glass needles injected various foreign substances into their tail fins, and kept the tadpoles under continuous observation for hours, days or weeks depending on the experiment.

Their results show that different cells respond differently to varying stimuli. Though all of the four types studied orginally were of mesenchymal origin, yet when differentiated into endothelium, leucocytes and connective tissue cells each type has a specific reaction and each cell remains specific. In all their work they never found any evidence of one type of cell changing into another type of cell. Several times they noted cases of apparent change, but continued observation proved there never was any real change. Each cell when once differentiated remained specific, no matter what stimuli were present.

For successful experiments on tadpoles' fins the substances must be readily injected through small glass cannulae which cause a minimum of injury, and the substances must remain unabsorbed long enough to be observed. The substances used were suspensions of carbon and carmine granules, droplets of an inert oil (paraffin), nutritive oils (fatty oils), aseptic oil, starch granules and similar substances in varying forms. These varying substances called forth varying responses among the several types of cells.

Connective tissue cells took up carbon and carmine granules and phagocytized them, but connective tissue cells did not touch starch or fat. These cells showed varying degrees of injury when an aseptic oil was injected.

Lymphatic endothelium grew towards fatty substances, but not towards starch grains.

Leucocytes responded with varying intensity to different substances. Their response to paraffin oil was no greater than their response to the injection needle. A few wandering cells appeared, then left the spot. Carbon, carmine and uncooked starch did not have much more effect. They attracted wandering cells and an hour or two later some leucocytes came from the nearby blood vessels and began to phagocytize the granules which remained as cell inclusions indefinitely. The control reaction to small glass tubes was very similar.

Fatty substances attracted more leucocytes. Clear leucocytes came to globules, ingested them, wandered to the lymphatics full of granules of fat, discharged them in soluble form into the lymphatics and came away clear. The rate of attraction for leucocytes depends upon the fineness of physical state. Fine emulsions of egg yolk and cream were most rapidly absorbed, dissolved and carried to the lymphatics. Olive oil globules were much more slowly absorbed by leucocytes. Lymphatic capillaries sent sprouts to them. This growth of lymphatic capillaries was especially noticeable in the case of slowly absorbed granules. With the fine emulsions, leucocytes acted before the lymphatics had shown much response. Feissinger and Marie claim to have discovered a lipase in lymphocytes and a protein-splitting enzyme in polymorphonuclears. The Clarks believe this conclusion may be borne out by the fact that often leucocytes near the fat remained unaffected, while others further away moved towards the fat. The experiments with fat have an additional interest in connection with the much debated question of fat absorption in the body. Why couldn't the leucocytes in the intestine absorb some fat and take it to the lymphatics? In the tadpole's fin the fat globules attracted or were positively chemotatic to lymphatic endothelium and to some leucocytes, presumably to the lypmhocytes.

When a microinflamation was caused by the injection of croton oil an extremely interesting reaction followed. All the cells in the region showed signs of injury. Due to granulations in epidermal and connective tissue cells, an opaque zone extended around the oil globule. All endothelial and connective tissue cells became swollen, and vacuolated, and the nuclei became very distinct. In addition connective tissue cells lost their processes and became shadowy, lymphaic sprouts within the zone were retracted and blood capillaries within the zone suffered from hemorrhage. In the blood vessels without the zone quickened flow was taking place and diapedesis. These leucocytes came to help the wandering cells of the neighborhood which had at once rushed to the injury. Leucocytes came from rather distant blood vessels to the site of injury. What interested us most was the almost uncanny way in which the leucocytes seized the shortest cuts to the injury, dodging connective tissue cells, and anything else in their way. Qute a number slipped into nearby lymphatics, slid to the tip, and then crawled out. They formed a sessile circle a little distance form the globules and sent out processes at first fine and transitory, but which soon became thicker and permanent, until the leucocytes resembled fibroblasts or connective tissue cells. This circle evidently localized the infection, because cells distal to it recovered at once. A different kind of leucocyte than is found in humans, the pigmented leucocyte, a very resistant type, next came upon the scene and boldly went up to the injection mass, actually flattening out against it. Soon there was such an accumulation of fluid at the spot that the injurious mass was forced out. As soon as the stuff was extruded, the tissue healed and the pigmented leucocytes wandered away. The clear leucocytes lost their processes, rounded up and finally, with regained amoeboid movement, wandered off. Connective tissue cells, stellate once more, came in to fill the gap where the oil had been. Within two days circulation was normal.

In their latest set of experiments (Oct., 1922), the Clarks worked with polysaccharids. They found so much difficulty in forcing grains and agar agar, etc., through the injection needles, that they tucked the needle into the skin, broke off the tip and left a small test tube open at the ends of the fin. The reaction caused by the needle was transitory and by the use of control experiments could be discounted. The reactions of the substances in the tube could be readily noted.

In many larvae tubes of cooked starch were injected into one fin, and tubes of uncooked starch were injected into the other. Leucocytes wandered to both, but many more leucocytes wandered to the cooked. Leucocytes filled the lumen of the cooked and all of the starch was inside the leucocytes. In the case of the uncooked, a few entered the lumen of the tube and ingested the refractile granules. In a week or ten days the tube was extruded. Some leucocytes with refractile granules still remained in the original situ, others wandered around in the nearby tissues still with refractile granules unchanged. Probably the cellulose covering prevented the action of the enzymes in leucocytes and lymph from attacking the granules which remained as cell inclusions (like carbon or paraffin).

In some experiments boiled starch alone was injected. In one case three granules were injected. In ten minutes they were but shadows. In sixteen minutes they had completely disappeared. An iodine stain showed a diffuse blue where the granules had been. Here the starch had dissolved before the tissue cells were visibly affected and before any leucocytes reached the

spot. Evidently tissue fluid is capable of dissolving and digesting cooked starch grains. Where more granules were injected, leucocytes aided the tissue fluids. Such a specimen stained with iodine showed two large blue granules with leucocytes about to engulf them, a finely granular blue stain and several leucocytes with bluish cytoplasm. An hour later no trace of blue was visible on staining with iodine.

Another set of experiments with gelatinized or semi-cooked starch called forth the most violent response of leucocytes. No other substance caused so many leucocytes to respond or to respond so quickly. The leucocytes rushed from the nearest blood vessels. Where semi-cooked starch had been was within fifteen minutes a dense mass of leucocytes. In one experiment, four semi and six completely cooked granules were injected. The leucocytes made a bee line for the former, actually passing between two cooked granules to reach a semi-cooked. Meanwhile the cooked granules became shadowy. As soon as the semi-cooked were disposed of, the leucocytes attacked the remnants of the cooked starch and finished digesting them. Fixed specimens showed that both polymorphonuclears and mononuclears were attracted by the starch. Iodine staining showed semi-cooked starch is ingested and dissolved by leucocytes (diffuse blue) and after three hours is changed to another substance, probably a colorless dextrin or sugar. (Tests to prove the presence of sugar were not successful. But this is not so significant, since injected solutions of dextrin gave negative sugar tests.) The experimenters compared the fate of the starch injected subcutaneously with the fate of starch treated with ptyalin and found that the tissue fluids acted more slowly and probably formed a colorless dextrin.

With agar agar, celloidin and gelatine, the leucocyte reaction is similar in intensity to that caused by the semi-cooked starch. As with starch, these colloids caused no response of connective tissue cells or of endothelium.

In all these experiments we saw how different cells phagocytized different foreign bodies. Usually the leucocytes were the most active phagocytes. Why the phagocytes should be attracted to the foreign body has proved a very baffling problem. The present trend of opinion is to regard it is a result of surface tension. In '90, Grabitchewsky classified substances as positively, negatively or indifferently chemotatic to leucocytes. Positively chemotatic substances being those which lowered the surface tension of the cell, causing the cytoplasm to flow toward them. In '21 Fenn worked out by calculus, physics, etc., the degree of intensity of attraction of various substances. He concluded it was a surface tension phenomenon. When he studied the reaction of leucocytes to quartz and carbon, he found that the carbon was phagocytized three or four times more rapidly than the quartz. Fenn claimed that the difference in rate was due to the more unstable condition of the carbon suspensions. A number of experimenters claim that they can duplicate all the ordinary phenomena which we may note in phagocytosis with a drop of chloroform in water, and they ascribe them all to surface tension. It seems as though we have no alternative but to accept some sort of physical explanation like this. Should we refuse we must then endow each individual phagocyte with reasoning powers that would do honor to any man.

Just as some philosophers would reduce each human being to a mechanical entity and explain all our actions by laws of physics and mechanics, while others see in each human being a miniature god, so in phagocytosis, one set of experimenters would endow each phagocyte with almost human psychic

powers of selective working—while others would reduce their whole action to the physical laws of tension.

Even as a few centuries ago, the development of that simple instrument, the telescope, brought far distant units of the universe to our ken, so with the intelligent use of labroatory technique, especially the microscope, within the last few decades, experimental biology has brought to our comprehension the equally distant and unknown universe of the actions and reactions of the cells of the body.

In the same way as physical laws, such as gravitation, explain nearly all the laws of the stellar universe, it is apparent more and more that physico-chemical laws, such as surface tension, explain most of the reactions of the foreign bodies to this cellular universe.

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THE EVOLUTIONARY SIGNIFICANCE OF VARIATION

EVOLUTION signifies a state of becoming. It does not necessarily imply that species have been getting better and better through the ages but rather that in accordance with the 'Law of Adaptive Radiation" they have been modified to meet the demands of their changing environment. Although little more than half a century has passed since the publication of the "Origin of Species" there has been welded a chain of evidence so complete that to-day few things in natural history have been more firmly established than the fact of evolution.

Students of comparative anatomy have shown the relationship between various groups of organisms and have clearly pointed out that structures which at first glance seem to be totally different are fundamentally the same. For example, if the foreleg of a lizard, the wing of a bird, the wing of a bat, the flipper of a whale and the human arm be carefully dissected, and the bones, muscles, vessels and nerves compared it will be found that the plan of structure is the same throughout and is merely modified in each case according to the use to which the part is put.

The paleontologists have examined the fossils of the plants and animals entombed in the rocks of the earth's crust and have dug up some of the most convincing proof of evolution. They have found that the oldest portions of the earth's crust are devoid of fossil remains. Either there were no living organisms or else they were of such simple structure that they left no record. The next geological era, the palaeozoic, marks not only the rapid development of the invertebrates but also the rise of the more primitive back-boned animals. This is followed by the mesozoic, the golden age of reptiles. Before the end of this era the primitive bird and first mammal have appeared. The cenozoic brings with it the high development of birds and mammals. The primates arise and finally man appears. Layer by layer the fossils tell the story of a gradual development from the simple to the complex.

The evidence offered by the embryologists is no less significant. Haeckel has summarized the contribution in his biogenetic law, namely, that the individual in his development recapitulates the history of the race. It must be remembered, however, that this history is greatly abridged. Structures are found in the human embryo which are typical in lower forms. These are transitory and are later either lost or greatly modified. In some instances, however, they remain and are found in the adult as vestiges. At a certain stage in the development of the human embryo gill-pouches are present. There is a two-chambered heart and a fish-like circulatory system. At a later stage the gill-pouches are obliterated with the exception of one which is converted into the Eustachian tube. The embryo of the whale has a dense covering of hair which is later lost, only a few coarse bristles persisting on certain parts of the adult body.

The geological distribution of animals and plants over the surface of the globe first turned the thoughts of Darwin towards evolution. All of the

evidence goes to show that each species has originated in a particular area and has spread from that point. In general, the longer the time any two regions have been separated, the greater is the difference between their flora and fauna. Thus the separation of Great Britain from the Continent took place in comparatively recent times and the animals found in both places are very similar. On the other hand, areas like Australia that have been isolated for a long time have their own peculiar species. On the island-continent are found the egg-laying mammals and nearly all of the known marsupials.

The latest link in the strong chain of evidence has been contributed by the serologists. A number of workers, notably Dr. George H. F. Nuttall, have shown that by means of the precipitation test the relationship among animals can be clearly demonstrated. Their results have checked up with those of the comparative anatomists and paleontologists. For example they have found that if the sera of the various apes be titrated against an anti-human serum, a good precipitate will be obtained with that of the higher anthropoids, a very slight one with that of the New World monkeys and none at all with that of the lemurs. The fact that apes are susceptible to human diseases further indicates a blood relationship.

The primary factor in evolution is obviously variation. Were no changes to occur, movement in one direction or another would be impossible.

The two main problems associated with variations are:

(A) The origin of variations, and

(B) The mechanism of their transmission.

At the outset we must recognize the fact that there are different kinds of variations. Some of them cannot be transmitted from parent to offspring and therefore play no part in the process of evolution.

We may classify all variations under two general headings:

1. *Congenital.* A better term would be "blastogenic," which signifies "present in the germ plasm." This type of variation is therefore intrinsic in the individual and is presumably not due to any external influence. It may appear, however, at any time from the beginning of embryonic existence to the death of the organism.

2. *Acquired.* In contrast to the preceding there are variations which are definitely extrinsic in nature and which are the result of external influences upon the organism. These, in as much as they are not inherent in the germ plasm, cannot be inherited by the offspring.

The last statement is accordance with the opinion of the vast majority of biologists.

It is important to remember that characters may be acquired "in utero" and that these are not necessarily congenital. An example of a congenital variation is the occasional occurrence of supernumerary digits in man. On the other hand the loss of a digit through amputation illustrates a variation that is acquired and therefore has no significance in race-history.

The group of congenital variations may be further divided into two classes:

a. *Continuous.* These are small, numerous and occur in a graded series. The increment of change between successive generations is extremely slight. Darwin called them "fluctuations" and thought that it was through the action of the Law of Natural Selection upon them that evolution had come about. Some workers, notably Prof. Karl Pearson, have applied the laws of mathematics to the study of the occurrence of variations. They have shown that

if a certain character, as height in man, be examined in a great many individuals and a curve plotted, it would conform very closely to the mathematical curve of probabilities. Over 4,000 individuals were examined and their measurements recorded by the Cambridge Anthropometric Society. A symmetrical curve was obtained and the numbers of departures from the normal, which in this case was found to be 69 inches, was the same in both directions. For example the number of men 67 inches in height equalled the number of men 71 inches.

b. *Discontinuous.* These are also known as "sports" or "mutations." They represent differences of great amplitude and, compared to the other type of variations, occur rarely. One of the classical examples is that of the short-legged ram which suddenly appeared on a farm in Massachusetts and which gave rise to the so-called Ancon breed of sheep.

Galton explains the formation of a mutant by comparing it to a polygon that changes its base. If the new bases becomes the one represented by the

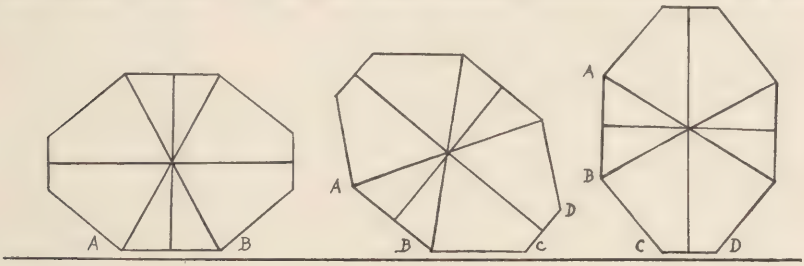


Figure 1.

line *B-C* it is unstable and will soon return to the old base. This would represent the ordinary continuous variation. If, however, a sudden force pushes it over to the base *C-D* it becomes a new form which is stable and which will not revert. This would correspond to a mutation.

De Vries divided mutations into three classes:

1. *Progressive.* The appearance of new characters. The character when first formed may be latent and may not appear in the individual for a number of generations.

2. *Degressive.* A sudden change from latency to activity.

3. *Retrogressive.* The converse of degressive. An active character that has suddenly become lost.

Jennings, after very careful work chiefly with animals that arise from a single parent, has attacked the conclusions of the mutationists. He claims that minute heritable variations—so minute as to represent practically continuous gradations—occur in many organisms and that these have been overlooked by the mutationists. These numerous small variations, he states, are acted upon by Natural Selection in accordance with the typical Darwinian scheme.

A. The question as to the origin of variations is by no means settled. The answer at best is a matter of speculation. The three principal explanations that have been offered are: (1) Inherent tendency; (2) Amphimixis; and (3) Mutations.

1. *Inherent Tendency.* Some believe that there exists in the germ plasm a force which causes it to constantly vary, thus bringing about variations. These may be fortuitous, fluctuating or follow certain fixed lines.

2. *Amphimixis,* or biparental parentage. The union of the traits of two lines of ancestry makes possible different combinations of characters. This principle which is advocated by Weissmann, may explain how recessive characters appear but leaves unexplained the origin of characters that never before have been present in the germ plasm.

3. *Mutations.* The sudden appearance of new types through a rearrangement of the chromosomes.

B. The second of the fundamental questions concerning variations, namely, the method of their transmission, may now be approached. In this connection four theories, those of Lamarch, Darwin, Weissmann and Mendell stand out conspicuously.

1. *Lamarck—The Inheritance of Acquired Characters.* This theory involves the principle that changes in organs brought about by use and disuse are transmissable to the offspring. In the human body there are a number of vestigial organs which represent structures that were once useful. The degeneration of the digestive tract in intestinal parasites like the tapeworm is closely associated with the disuse of that organ. The Neo-Lamarckians of today claim that the germ plasm responds directly to the changes in the body, and these in turn go back to the conditions of the external environment. No experimental proof, however, has been offered to substantiate this theory. On the contrary there is abundant evidence that changes in the soma have no effect upon the germ-plasm.

2. *Darwin—Natural Selection* and *Pangenesis Theory.* The first theory is based upon the fact of universal variability. There being more organisms born than can possibly survive, a struggle for existence ensues among various groups and among the members in each group. The result. as Herbert Spencer has so aptly phrased it, is the survival of the fittest. Darwin added another factor as a corollary to his first theory, namely, that of Sexual Selection. In this way he explained the brilliant colorings of male birds and the probability of the strongest males reproducing their kind. In order to explain how the new variations got into the egg or sperm he devised the *Theory of Pangenesis,* according to which every cell in the body produces minute particles called "gemmules," which are of the same nature as the cells producing them. These gemmules which constitute a miniature replica of the parent's body finally congregate in the reproductive cells. This theory, which would allow of the transmission of acquired characters, has been shown to have no basis in fact.

3. *Weissmann—Germ Plasm Theory.* According to this theory only germ plasm can be transmitted. The somatoplasm is concerned only with the individual and plays no part in the perpetuation of the race. This theory therefore throws out of consideration the transmission of changes in the soma of an individual.

4. *Mendel—Segregation of Characters—Laws of Dominants.* Entirely independent of cytology Mendel worked out a number of laws which have been universally accepted. His theories are the result of the experimental breeding of plants. The Law of Segregation postulates the existence of unit character contributed by the reproductive cells of both parents. These do not blend but remain in their original purity and can be segregated. The second law affirms that unit characters are either dominant or recessive and will appear in sub-

sequent generations in accordance with the principle of the "Mendelian Ratio."

It is thus seen that proof for the method by which variations arise is far from complete.

All of the theories so far advanced lack conclusive evidence. This much, however, has been accepted. No variation is transmitted unless the germ plasm has been affected. In other words a rearrangement of the chromatin material must occur. Whether environmental conditions play any part is still a matter of dispute. It is conceded, however, that certain diseases, like syphilis, and possibly certain drugs, may directly modify the germ plasm. Once a change has occurred in the germ plasm, however, it will be transmitted in accordance with the laws of Mendelian Heredity.

In the dissecting and operating rooms variations in the human body are often met. These are not always the result of a haphazard development. They may be present as normal structures in other animals and their significance in the human can only be appreciated in the light of the evolutionary teachings of comparative anatomy. Man is but one rung, even though that be the highest. in the ladder of evolution. He belongs to the class of Primates and it is important that we glance hurriedly at the chief characteristics of the group. They are: (1) Chiefly aboreal animals living on fruits, (2) prehensile limbs with thumb and great toe shorter than the other digits and opposable, (3) plantigrade walking position of the feet, (4) terminal flattened nails instead of claws, (5) single pair of pectoral mammae, (6) brain large and convoluted.

According to the Classification suggested by Prof. W. K. Gregory, the group may be divided as follows:

Sub-Order 1. *Lemuroidea* (Lemurs or Half Apes). Most primitive. The cerebral hemisphere is so small that it does not cover the hindbrain. The second finger retains the ancestral claw. During the Eocene period they lived both in North America and Europe, thus showing their great antiquity.

Sub-Order 2. *Anthropoidea*

Series 1. *Platyrrhini* (New World Apes). Have broad nasal septum. Thumb is not opposable but usually reduced to a small vestige. The tail is long and prehensile.

Family 1. *Hapalidae* (Marmosets). Small squirrel-like monkeys with a long hairy non-prehensile tail. Have claws instead of nails.

Family 2. *Cebidae* (Capuchins, howler monkeys, spider monkeys). Have flat nails instead of claws and usually a prehensile tail.

Series 2. *Catarrhini* (Old World Apes and Man)

Family 1. *Cercopithecidae* (Baboons, mandrills, macaques). Quadrupedal habit of locomotion, dog-like heads, no vermiform appendix, omnivorous in diet, large canine teeth.

Family 2. *Simiidae* (Gibbon, orang, chimpanzee, gorilla). The man-like or anthropoid apes. Tail is rudimentary. Arms are longer than legs. There is a vermiform appendix. The hair is mainly on the ventral surface of the body and the limbs.

Family 3. *Hominidae*.

The primates arose probably about the dawn of the Eocene era. They as well as the carnivores, sprang from a common insectivore stock. The last-named group which included shrews, hedgehogs and moles, were very plastic and progressive mammals. In the Miocene era geologic changes caused the ancestor of man to come down from the trees—a step essential to further human progress. Continental elevation brought with it increased aridity of climate, especially to the north of the Himalayas. With this increased aridity and lessening of the tropical heat came the dwindling of the forests and the ape-man was forced to come to earth. Evidence points to Central Asia as the birthplace of mankind and to the Miocene era one to two million years ago as the time. Modern man, however, did not develop at once. Fossil remains discovered within recent years, have shown that tentative men were produced. There are no living representatives of these men today. The earliest of the prehistoric human species was *Pithecanthropus erectus*, who lived in the late Pliocene era about half a million years ago. He had a gibbon-like skull-top and a brain about two-thirds the size of modern man, a modernized human femur and subhuman upper molars. *Homo Palcanthropus Heidelbergensis*, who appeared in the Pleistocene age about 400,000 years ago, had a massive jaw that was more simian in character than human. He lacked entirely the chin prominence which is characteristic of modern man. The *Piltdown Man* (*Eoanthropus dawsoni*) also lived in the lower Pleistocene era in the second or third interglacial period. The skull which was found indicated a large brain and high forehead. *Homo neanderthalensis* had a big brain, great ape-like eye-brow ridges and massive jaws. He used fire and fashioned implements. He disappeared about the end of the fourth great Ice Age.

VARIATIONS IN MAN

Prof. Huntington's classification of the variations that occur in man will be followed in this paper. The variations are divided into two groups, ontogenetic and phylogenetic. In the former class there are placed these structures which occur normally in the embryo but are not carried into the typical adult organization. In the latter class there are grouped those structures which are not ordinarily found in the embryo but which either indicate a reversion to some ancestral type, in as much as they are found as normal structures in the ancestors of the species, or else mark progressive evolutionary changes.

1. Ontogenetic Variants

A. *Errors in Development:*

The majority of the ontogenetic variants naturally fall into this class. The fact that man in his development recapitulates his phyletic history means that structures will appear in the embryo which are either lost or greatly altered in the adult. Many of these early structures persist as vestiges in the normal body. The human body is a veritable museum containing many relics which at one time played important roles, but which today are of no use and merely serve to indicate man's line of descent. As Darwin himself put it, "Man still bears in his bodily frame the indelible stamp of his lowly origin." The "plica semilunaris" is a crescentic fold of membranes in the inner upper corner of the eye. It is well-developed in birds and in may mammals, as the rabbit, where it acts as a nictitating membrane and is used in cleaning the front of the eye. In man and monkey it has dwindled due to the fact that the upper eyelid has become much more mobile. Some men show on the in-

wardly turned margin of the ear trumpet a little conical projection. This is known as "Darwin's Point," and is a vestige of the tip of the pointed ear of the lower mammals. The pineal body of the brain is connected in reptiles, notably Hatteria, with a third eye, which really represents a primordial vertebrae eye. In man this is present as a vestige hidden beneath the mass of the fore-brain. The vermiform appendix, which in the herbivora is large and of high digestive value, the direction of the hair on the body, the grasping power of the infant, the strong inward deflection of the feet of the foetus together with the mobility and distinct projection of the great toe at an angle from the side of the foot which persist in the early months of extra-uterine life, all indicate man's relationship to the lower forms.

1. *Arrest of Normal Development.*

This is one of the most frequent causes of variations under the general heading of errors in development. An example is the hare-lip. The upper lip is produced by the fusion of three parts. If these fail to unite a cleft between the nostrils and mouth results. The only adult animals in which such a condition persists are the sharks and rays. Cleft-palate is often combined with hare-lip. This represents an arrest at a later stage of development. The parts of the upper lip are united before the end of the second month of foetal life but the right and left outgrowths which meet along the roof of the mouth to form the palate are not completely joined until the end of the third month. In amphibia, reptiles and birds the three parts representing the upper lip unite but a cleft palate remains. This condition may therefore be considered reptilian in character.

2. *Failure of Normal Development.*

Sometimes a muscle or group of muscles fail to develop. A child 4 months old, was admitted to the Presbyterian Hospital (case No. 22,195), was found to be suffering from a congenital absence of pectoralis major and minor, intercostal and triceps muscles. Since birth the child had kept the arms strongly flexed and the hands extended. If the arms were straightened out forcibly they assumed the flexed position as soon as the external force was removed.

3. *Atypical Development of Vestigal Structures of a Transitory Character in Normal Development.*

The aorta may arch over the root of the right instead of the left lung, a condition that is normal for birds. In such a case the aorta descends on the right side of the vertebral column. A transposition of the other viscera is commonly associated with such an arrangement. Less frequently the aorta, after arching over the root of the right lung, runs down the left side of the vertebral column. This latter peculiarity is not accompanied by a transposition of the viscera. This may appear at first glance to be a reversional character referable to the avian type and thus show a relationship between bird and man, but there is abundant proof to the effect that mammals did not develop from the birds.

Sometimes the aorta subdivides soon after its origin into two branches which soon reunite. This is the normal condition of the vessel in the reptiles.

4. *Atypical Development of Permanent Vestigial Structures.*

Man has thin bands of muscles which are arranged to move the ear, and these are normally functionless. Probably when man assumed the erect posi-

tion with eyes looking forward the head became more movable and rendered unnecessary the moving of the ear to aid in the appreciation of sound. In some individuals the muscles retain their original function, a condition analagous to that in the lower apes and quadrupeds.

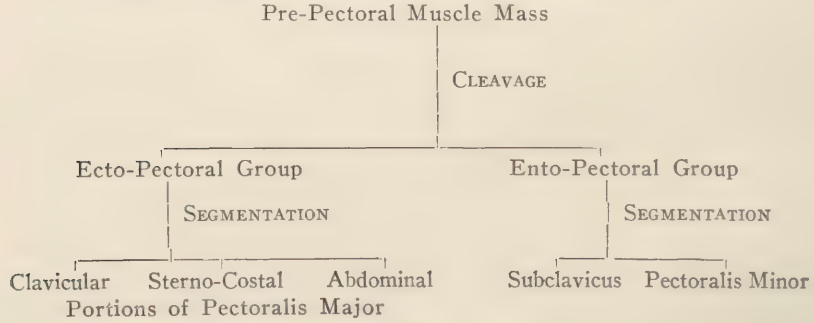
5. *Errors in the Definition of Muscular Integers.*

In order to appreciate the errors that may take place in the definition of muscle integers, it may be well to briefly review the normal embryological development of these units. The skeletal muscles, with the exception of those connected with the branchial arches, are derived from the myotomes of the mesodermic somites. The fibres of each myotome are at first loosely arranged. Later they become more compact and are then arranged parallel with one another, their long axis running antero-posteriorly. Then one or more of the following changes occur:

1—Cleavage
2—Segmentation
3—Migration
4—Metamorphosis
5—Fusion

The first three may be illustrated by the development of the pectoral group of muscles. Lewis describes the Pectoralis Major and Minor and the Subclavius as arising from a pre-muscle mass which is located in the lower cervical region on the medial side of the arm bud, anterior to the first rib. The mass thus lies at first in the region of its nerve supply, receiving branches from the sixth, seventh, and eighth cervical and first thoracic nerves which proceed almost directly lateral. This mass, as development proceeds, moves from its ontogenetic point of origin to the position obtained in the adult. This is known as *migration.* In the 14 mm. embryo, the caudal end of the muscle mass has extended to nearly the tip of the fifth rib and the fibres arise from the upper five ribs and the anlage of the sternum and clavicle. At the same time, through the process of *cleavage,* the mass has split into a superficial or ecto-pectoral group which will become the three parts of the Pectoralis Major, and a deep or entopectoral group, which will split into the Pectoralis Minor and the Subclavius. In the 16 mm. embryo, the Pectoralis Major extends to the sixth rib and shows a differentiation between the costal and clavicular portions. This splitting of the ecto-layer into distinct parts (clavicular, costo-sternal, and abdominal) is known as *segmentation.* By this same process the ento-layer divides into the Subclavius and the Pectoralis Minor.

These changes may be graphically represented as follows:



In *metamorphosis* we have a degeneration of muscle tissue into fibrous and ligamentous tissue. This change may affect either the whole myotome or a portion of it. Many of the aponeurotic sheets and ligaments of the human body are thus formed. The Lumbar Aponeurosis and the Aponeurosis of the Oblique and Transverse Abdominal muscles may be given as examples. In *fusion* parts of successive myotomes unite to form a single muscle. The Rectus Abdominis is formed by the fusion of the ventral portions of the last six or seven thoracic myotomes.

It is at these various stages in the development of the muscles that the errors may arise. It is important to note that while these variants are atypical in humans, they may be normal constituents of animals of other mammalian orders. We must constantly bear in mind the fact noted earlier in this paper, that the human embryo in its development repeats its phyletic history.

a. *Errors in Cleavage Into Successive Muscular Planes:*

An example of this would be the Tensor Semi-Vaginae Articulationis Humero Scapularis one of the deep supernumerary muscles in the ecto-entopectoral interval. When that muscle is present, there is a deficiency in the Pectoralis Major between the cartilages of the third and fourth ribs near the sternum. It passes laterally, distal to the Subclavius and Coracoid Process. It then expands into a strong triangular tendon, which continues beneath the ventral margin of the Deltoid and joins the deep layer of the Deltoid fascia, thus gaining an indirect attachment to the lateral humeral ridge and tuberosity. The fact that it corresponds to the deficiencies in the Pectoralis Major and that it is innervated by the Anterior Thoracic Nerves, indicates that it is a displaced segment of the Pectoralis Major. Although it retains its characteristic ectopectoral origin, it shifts its humeral insertion toward the Coracoid Process. It will be recalled that it is only in man and anthropoid apes that the Pectoralis Minor is inserted into the Coracoid Process. In the lower forms it is attached to the humerus. The insertion of the Tensor Semi-Vaginae does not quite reach the Coracoid Process, but terminates as a slip intermediate between the insertions of the Pectoralis Major and Minor. It thus obtains an indirect humeral attachment through the Subdeltoid Aponeurosis.

The Sterno-chondro-humeralis, which is a normal constituent of the pectoral musculature of some of the lower mammals, corresponds in all essential features to this human variant and may therefore be considered its homologue.

b. *Errors in Segmentation Into Components Within the Confines of a Single muscular plane:*

As an illustration of a muscle formed by such an error, we will take the Pectoralis Quartus. This muscle which, when present, is usually associated with the Axillary arch, represents as Prof. Huntington writes, "an incomplete stage of that differentiation from the Ventro-appendicular muscle sheet which we must regard as the final step in the production of the general panniculus." The Panniculus Carnosus is the skin-moving muscle of the lower mammals and is used by these animals as a protection against the bites of insects. It is a thin, flat muscle, found directly under the skin, spreading over the side of the body of the animal and in some cases extending to

the mid-dorsal and mid-ventral lines, incasing the trunk in a subcutaneous muscular sheet. It is present in the Monotremes and in many of the other forms of mammals, but is normally absent in man. It occasionally manifests itself in man in the form of the Pectoralis Quartus and the Axillary Arch. The Panniculus is frequently encountered as in Rodents, in two layers—a superficial and a deeper layer. The latter is intimately connected with the Pectoralis Major. The Pectoralis Quartus belongs to this deeper layer—its segmentation from that part of the Panniculus being incomplete.

In as much as the Pectoralis Quartus is a rudiment of a muscle normally found in mammals, it has a phylogenetic significance and may be regarded as a progonal variant. The same may be said of the Tensor Semi-Vaginae whose homologue is found in lower mammals.

c. *Errors in Migration:*

An example of this type of error is the much-discussed Sternalis muscle which is present in about 4% of persons. It is a small muscle which lies in front of the thorax parallel with the sternum. It arises from the aponeurosis of the External Oblique and the lower true ribs and cartilages and inserts into the sternal tendon of the Sterno-Cleido-Mastoid or fascia over the Pectoralis Major. It may be present in one of three types:

1. Well-developed Sternalis with a corresponding deficiency in the sterno-costal portion of the Pectoralis Major.

2. Slender and delicate ribbon-like Sternalis overlying a normal Pectoralis Major having no deficiency.

3. Sternalis directly connected with the Pectoralis Major, the muscular fibres of both being continuous, or the latter taking origin from part of the former.

Several theories have been advanced to explain the presence of this muscle. Some believe it to be the downward prolongation of the Sterno-Cleido-Mastoid, others that it represents the upward prolongation of the Rectus Abdominis. Turner considers it a reversional part of the Panniculus. Cunningham and Eisler call it a displaced and rotated segment of the Pectoralis Major. They emphasize the fact that both muscles are innervated by the same nerves (Anterior Thoracic). Of these various theories, the last one seems to be confirmed by embryological findings. According to Lewis the pro-pectoral mass during its ontogenetic migration laterally and caudally, divides into a deep and superficial plane. The deep pectoral plane becomes attached to skeletal points and is arrested at an early period. The superficial fibres, however, continue to descend and soon overlap the deeper layer. While these superficial fibres are still free, some etiological force may cause its superior portion to become attached to the sternal tendon of the Sternal-Cleido-Mastoid, or else it may become included in the closure of the sternal bars in the mid-line during the formation of the adult sternum. The superficial fibres continue to migrate caudally, swinging the attached portion into a vertical position, and we have a Sternalis. If a large part of the Pectoralis Major becomes fixed, a Sternalis of type results. If only a small part of it is affected there is no deficiency in the muscle and we have the type 2 variant. Type 3 occurs in cases in which the separation is not complete.

d. *Errors in Metamorphosis:*

The lesser sacro-sciatic (sacro-spinous) ligament is intimately associated

with the ischio-cocygeus muscle and it is regarded as having been derived from it by fibrous transformation of the muscle fasciculi. The transformation may not be complete and part of the ligament may be made up of muscle fibres.

B. *Reversional Ontogenetic Variants:*

These variants appear normally in the embryo but are usually lost in the course of later development. If they persist, they have a reversional significance because they can be referred back to normal structures in lower animals. An example of such a variant is that of thirteen free ribs, occasionally found in man. This condition is normal for anthropoid apes with the exception of the orang. An interesting human case was described by A. Low in the Journal of Anatomy and Physiology (vol. 34). The subject was a male. He had thirteen pairs of ribs of which eight joined the sternum. The thirteenth rib on the right side was 41 mm. long and that on the left side 69 mm. They articulated with the twentieth, or first lumbar, vertebra. In this specimen the first sacral was not fused with the other sacral vertebrae, a condition which is frequently associated with thirteen ribs. In other words, the forward migration of the pelvis had been arrested at the twenty-sixth vertebra. Wiedersheim states that the thirteenth rib, which appears in the embryo, begins to degenerate as soon as the twenty-fifth vertebra is incorporated in the sacrum.

At the Orthopaedic Hospital there are some twenty cases on the current record of variations in the number of ribs. A few of them are of the reversional type described above. If the variation is asymmetrical, there is apt to be asymmetry in the vetebral body formation. A case is that of F. J., female, age 3½, who had a slight deviation of the spine to the right. It was found that there were 13 ribs on the right side and 12 on the left.

Another reversional variant in the adult human which is normal is the embryo, is supernumerary mammary glands. In the six-week embryo there appears a thickening in the ectoderm extending along the body wall from the axilla to the groin. This is the primary mammary ridge or milk line. If more than one pair of mammae persist in the adult, the condition is comparable to that in the lower mammals. Two such cases are on record at the Roosevelt Hospital (B-16,126—a female, 45, having an additional mammary gland in the upper and outer quadrant of the left breast extending towards the axillary fold; and A 14,080—a negro male, 51, having an accessory gland in the skin of the right axilla).

C. *Progressive Ontogenetic Variants:*

A tendency to the disappearance of more ribs in man is seen occasionally in the loss of the twelfth rib as a free skeletal segment through its synostosis with the nineteenth vertebra. There are cases at the Orthopaedic Hospital which show an intermediate step in the disappearance of the twelfth ribs. The ribs are still present as free skeletal structures but are greatly reduced in size. There are other cases in which they have entirely disappeared as free segments and are present merely as enlarged transverse processes of the vertebrae. A case of the intermediate type is that of C. A., male, age 24, who had twelfth ribs which formed joints with the nineteenth vertebra, but were only 1½ inches long. A case in which complete disappearance had occurred is that of A. M., male, age 27, who had 11 pairs of ribs and 6 lumbar vertebrae.

2. Phylogenetic Variants

A. *Reversional Phylogenetic Variants*:

In this class of variants will be found structures that do not appear normally in the embro, but occur typically in lower animals. They are divided into 3 groups: (1) archaeal—those that can be referred back to the promammalian forms; (2) progonal,—those that go back only as far as the mammalian stem, and (3) ataval,—those that are present in the primate organization.

1. *Archaeal Group* consists of variants which go back the furtherest in man's phyletic history. These appear as typical structures in the ancestors of the mammals. An example is the ent-epicondylar foramen (Plate I), an opening on the inner side of the lower end of the humerus. It is found nearly always in fossil reptiles. The functional significance of the foramen is probably the protection of the brachial artery and median nerve, which pass through it, against severe pressure in the forcible flexion at the elbow joint. It was therefore widely distributed among the active and powerful early reptiles, but it has been lost in their creeping descendants, with the exception of "Hatteria punctata." To-day it is found variously distributed among mammals, especially in those forms that use their anterior extremity extensively. The foramen is not present in birds.

Progonal Group: In this class are placed the variants which occur

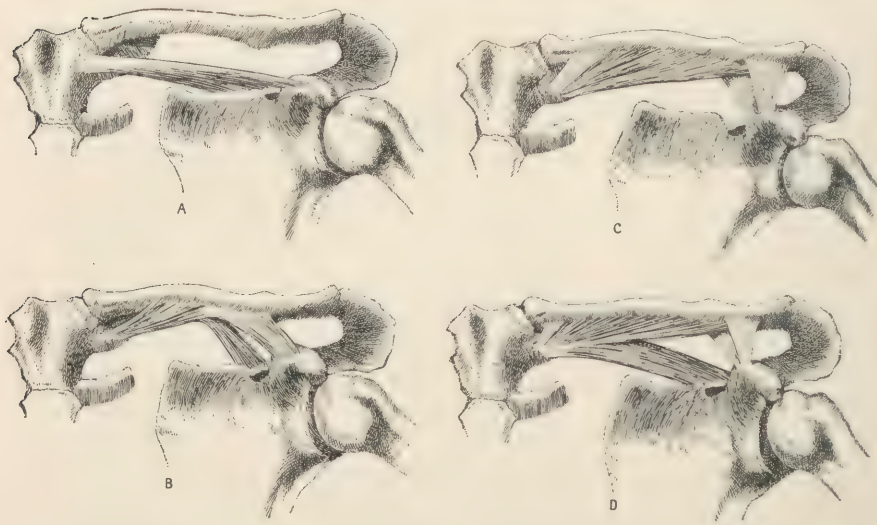


Figure 2. Schemata showing the effect of the introduction of the clavicle on the disposition of the M. Sterno-costo-scapularis.

A. Primitive condition. M. Sterno-costo-scapularis, as it occurs in non-claviculate mammals and in the human variant, without attachment to the clavicle.

B. The central portion of the muscle acquires a secondary attachment to the clavicle, resulting in the establishment of two derived muscles, the Costo-clavicularis and Coraco-clavicularis.

C. Typical condition in Man. Some of the proximal fibres of the Costo-clavicularis metamorphose into the Rhomboid ligament, the main portion of the muscle becomes the Subclavius and the Coraco-clavicularis furnishes the Conoid and Trapezoid ligaments.

D. Human variant. The normal Subclavius and Clavicular ligaments are associated with a Costo-scapularis.

as normal constituents in animals falling within the limits of the general mammalian organization. The Sterno-costo-scapularis muscle (Fig. 2 and Plate V) may be taken as an example of such a variant. This muscle is widely distributed in land-living non-claviculate mammals, such as the ungulates. It extends, in its primitive form, from the manubrium of the sternum and the first costal cartilage to the Coracoid Process and the adjacent cranial border of the scapula. In the lower claviculate mammals, as the Rodents, the muscle gets an intermediate attachment to the clavicle, resulting in the formation of two muscles: Costo-clavicularis and Coraco-clavicularis. In the higher forms, such as Man and the Primates, the proximal fibres of the Costo-clavicularis muscle have changed, through the process of metamorphosis into the Costo-clavicular (Rhomboid) Ligament, while the main portion of the muscle has become the Subclavius. The Coraco-clavicularis muscle has metamorphosed into the Coraco-clavicular Ligaments (Conoid and Trapezoid). As a variant in man, the Sterno-Costa-clavicularis muscle may appear alone, just as it does in non-claviculate mammals, or it may exist together with a normal Subclavius. In the latter case, it is referred to as "reduplication of Subclavius."

Ataval Group: These variants go back to a more recent ancestry than either of the other two groups. A good example is the default of the Peroneus Tertius, a condition which occurs quite frequently. In Man, the Tibialis Anterior and Posterior tend to invert the foot. This is the condition found normally in apes. These animals are thus compelled to walk upon the lateral border of their feet. This action is antagonized by the Peroneus Longus and Brevis. The latter two muscles, however, are not sufficient to overcome the inverting power of the Tibialis muscles. Hence, a new element appears in man to aid in neutralizing this inverting tendency. This additional muscle is the Peroneus Tertius which is formed from the distal portion of the Extensor Digitorum Longus. The three Peronei muscles, now acting together counteract the effect of the invertors and Man is thus able to walk upon the plantar surface of his feet. His fully erect position is thus made possible. The Peroneus Tertitus is a distinctly human muscle and is absent even in the Anthropoid Apes. Its absence in Man is therefore a reversion to the Primate Type. Recently an incompletely developed Peroneus Tertius was found by Prof. Huntington in a male gorilla. This constitutes a progressive variation for the ape.

Normally in the lower primates, instead of a Peroneus Tertius there is an Extensor Quinti Digiti Brevis which arises from the fibula in close relation to the Extensor Digitorum Longus. Its tendon descends in company with the Peroneus Brevis and is inserted on the lateral side of the long Extensor tendon to the fifth toe. There is occasionally found in a human subject a Peroneus Brevis which, before reaching its normal insertion into the base of the fifth metatarsal, gives off an additional tendon which passes to the Long Extensor tendon. This would likewise be a reversional character, but it would be progonal in nature because it may be traced back to the common mammalian stem. It illustrates a condition which is normal in the Monotremes.

The Peroneus Tertius itself may sometimes send an additional tendon to the fifth, or even the fourth toe, thus betraying its primitive derivation from the Extensor Digitorum Longus.

The humeral insertion of the Pectoralis Minor is another illustration of an ataval variation.

b. *Progressive Phylogenetic Variants*

These illustrate the evolutionary processes which are at present active in man. Characters appear which perhaps some day will be part of the normal human structure.

1. *Advance in the Vertebral Level of the Pelvic Girdle.*

In the evolution of man and the higher apes there has been a shortening of the longitudinal body axis due to the forward shift of the pelvic arch. The normal adult presacral portion of the vertebral column contains 7 cervical, 12 thoracic and 5 lumbar vertebrae. Thus the 25th vertebra is normally the first sacral element. The synostosis of the sacral segments has gone in a caudio-craniad direction. Man bears evidence of this in the fact that the second sacral vertebra is less completely fused with the first than it is with the third. In man the advance of the pelvic arch normally terminates when the 25th vertebra becomes incorporated in the sacrum. In some cases the number of presacral vertebrae is reduced to 23 (Plate II). This is a further advance in pelvic migration and is of progressive significance because it anticipates a change that has not yet occurred in the great majority of the species. This advance may be on one or both sides. The opposite condition sometimes occurs. The presacral vertebrae is increased to 25 (Plate III). In this case the pelvic advance is retarded and the variant is of a regressive type.

It is interesting to note that in the three higher anthropoid apes, Orang, Chimpanzee and Gorilla the pelvic migration has been carried one segment further than in Man. This may be interpreted as an attempt to counteract the inadequacy of visceral support by the pelvis, especially the lateral expansion of the ilium. In the Gibbon, on the other hand, the pelvic shift has been arrested one segment further caudad than in Man. Thus, as far as pelvic advance is concerned, Man occupies an intermediate position between the higher Anthropoids and the Gibbon.

Cases of sacralized 5th Lumbar vertebrae in Man are not uncommon. The following cases were obtained at the Orthopaedic Hospital. No. 56,542—Male, 32—Back and right hip painful. Marked limitation of motion in the lower lumbar region. Some limitation of lateral bending to the left, more to the right. X-ray showed sharp lumbo-sacral angle. Transverse processes of 5th Lumbar in abnormally close relation to the wings of the sacrum.

No. 52,940—Female, 37—Pains in lower region of back for 8 or 10 years. X-ray showed that the lower lumbar transverse process on the right side was bifid, the lower horn articulating with the wing of the sacrum and the ilium. The left transverse process impigned against the wing of the sacrum. The spinous processes of the 4th and 5th lumbar appeared in abnormally close relation.

Operation—spinal fusion of 5th lumbar to sacrum.

2. *Congenital Absence of the Vermiform Appendix in Man.*

Instances of such a variant are rare. In the cases reported, the caecal outgrowth from the embryonic intestine develops only sufficiently for the establishment of the normal adult pouch (Plate IV). The distal portion, which would ordinarily give rise to the appendix, defaults. In as much as the appendix is merely a vestige in Man its disappearance may be regarded as of progressive significance.

3. *Progressive Peroneus Tertius.*

An interesting anomaly of the Peroneus Tertius (Fig. 3) was found in a Chinese male, age 54, in the Anotomical Laboratory of the Peking Union Medical College. In both legs the muscle had a fleshy origin from nearly

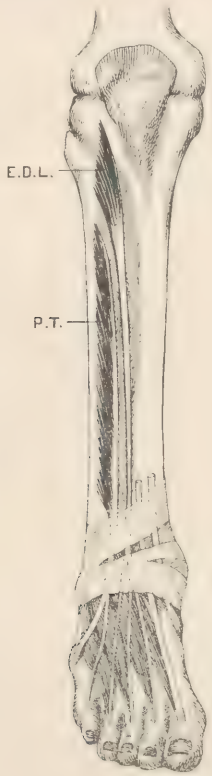


Figure 3. Semidiagrammatic drawing showing relations of anomalous peroneus tertius and extensor digitorum longus. Other anterior leg muscles represented by their cut tendons only. (After Stevenson.)

the entire length of the anterior part of the medial surface of the fibula. A very small Extensor Digitorum Longus had an origin in the proximal 5 cm. of the bone. There was a space of 3 cm. between the lower margin of the Extensor Digitorum Longus and the upper margin of the Peroneus Tertius. The margins of the muscles were well rounded and distinctly formed. The anomalous muscle gave rise to a strong tendon which passed beneath the transverse and cruciate ligaments and divided into two separate tendons which became inserted into the base of the fifth metatarsal and the first phalanx of the little toe. The small Extensor Digitorum Longus gave rise to a very slender tendon which began at the upper margin of the Peroneus Tertius and passed down the leg, under the transverse and cruciate ligaments, subdividing on the dorsum of the foot into three smaller tendons having their insertions on the proximal phalanges of the second, third and fourth toes. Branches from the deep peroneal nerve supplied both muscles.

The Peroneus Tertius is a distinctly human muscle being normally absent

in all lower forms. It is usually considered as a differentiated portion of the Extensor Digitorum Longus and its variants are commonly interpreted as mere variations in the degree of differentiation from this muscle. But this anomalous Personeus Tertius seemed to be much more than this. It had almost completely usurped the position of the Extensor Digitorum Longus without assuming a proportionate share of its function

The right calcaneus of this cadaver showed a striking deformity. The part of the bone bearing the main articular facet and receiving the greater part of the weight of the body through the talus was tipped laterally on its antero-posterior axis, indicating that the forces responsible for this impaction came while the foot was in a position of strong eversion.

It will be recalled that apes normally have no Peroneus Tertius and that they walk with their feet inverted. It is through this muscle that the human foot has attained its plantigrade position. In the variant just described the muscle has developed much further than in the normal individual and has strongly everted the foot. It is evident that in this case Nature has gone one step further, and thus this variant indicates what we may expect in the way of further evolutionary change in the human foot.

Conclusion

In conclusion we may state that evolution has occurred through the transmission of variations from parent to offspring. Just how these variations arose is by no means clear. Somehow or other the germ plasm was affected and a rearrangement of chromatin material took place. Once a congential variant arose it reproduced itself in accordance with the laws of Medelian heredity. If this variant were of a progressive nature and made its possessor better equipped than his fellows to adapt himself to the environment, it was very apt to survive and become incorporated into the general make-up of the species. The variations that occur in Man and that are not fortuitus may have an ontogenetic or phylogenetic interpretation. If of the former type they represent some stage of development which is normally present in the embryo. If of the latter type, they are not normally present in the embryo but are either found as typical structures in Man's ancestors or else are of a progressive type and indicate the probable path of future evolutionary change.

Whether Man is to climb still higher it is difficult to say. Some believe that the Law of Adaptive Radiation has ceased as far as Man is concerned and that any change now is largely retrogressive. They point to such changes as the reduction of hair and of teeth and the dulling of the senses of sight, hearing and smell. But evolution does not imply the constant improvement in function of a given organ. The reverse has often occurred in the evolutionary history of many species. However, if the evidence be carefully weighed and interpreted in the light of the phyletic history of the race, it seems very probable that variations of the type considered last point the direction to which Man is headed.

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PLATE 1

EXPLANATION OF FIGURES

1. Right and left humeri in a specimen of *Hyaena striata,* showing the occurrence of the entepicondylar foramen as a reduced variant on the right side.

2. Left humerus of *Felis leo,* showing entepicondylar foramen characteristic of the Felidae.

3. Series of adult human humeri with supracondylar process.

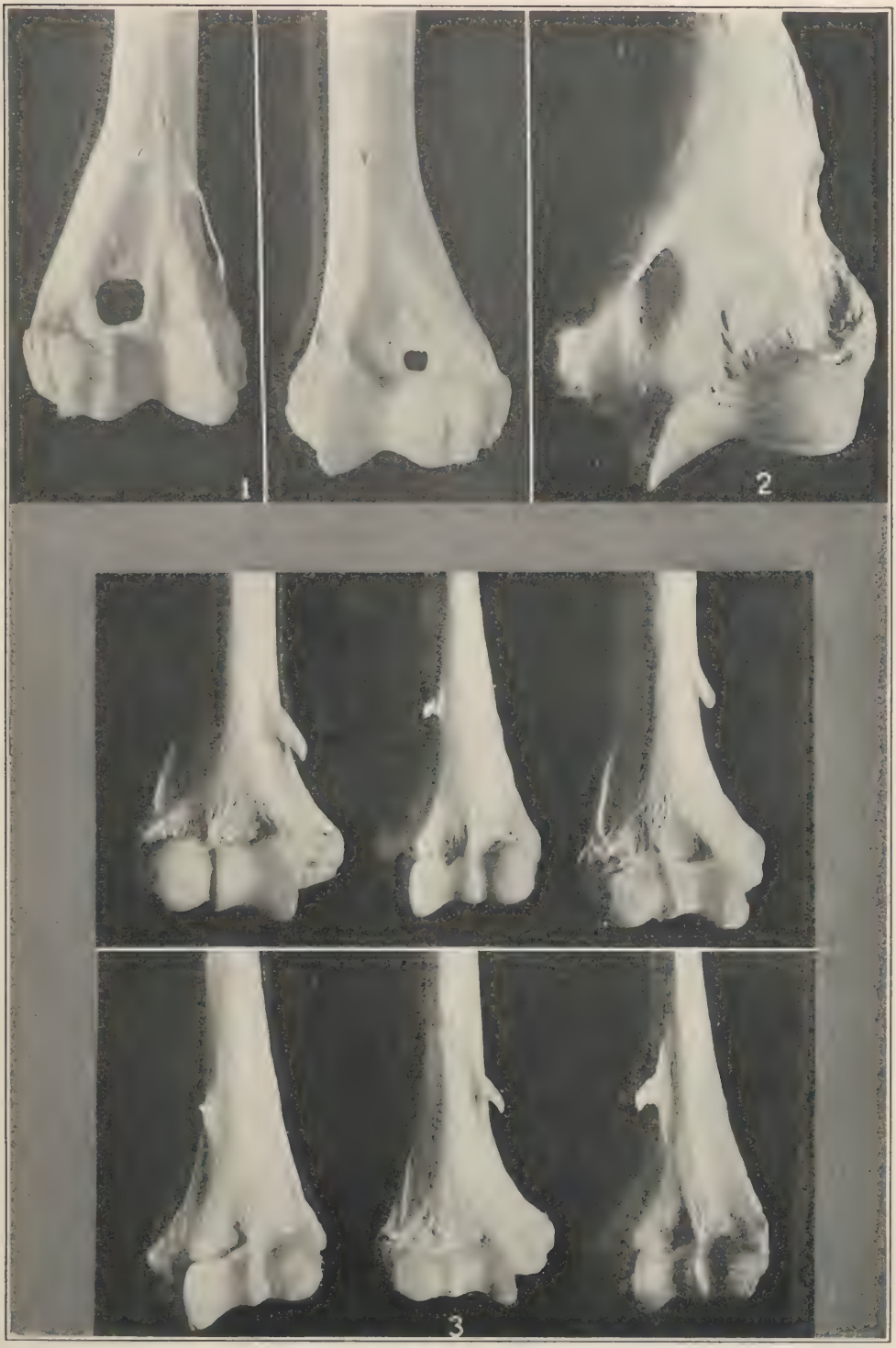
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PLATE II

Adult human instances of phyletic advance of pelvic migration.

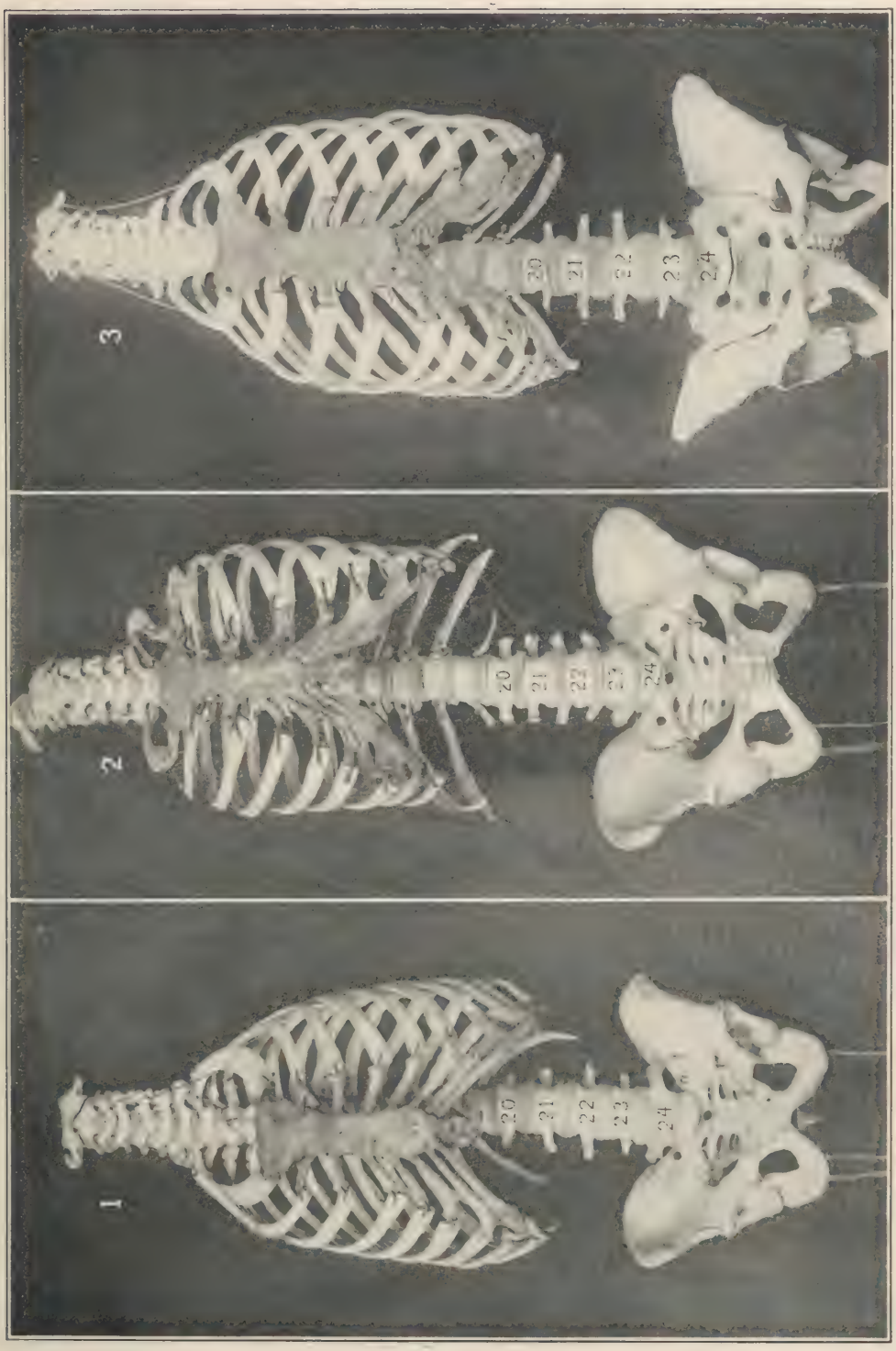
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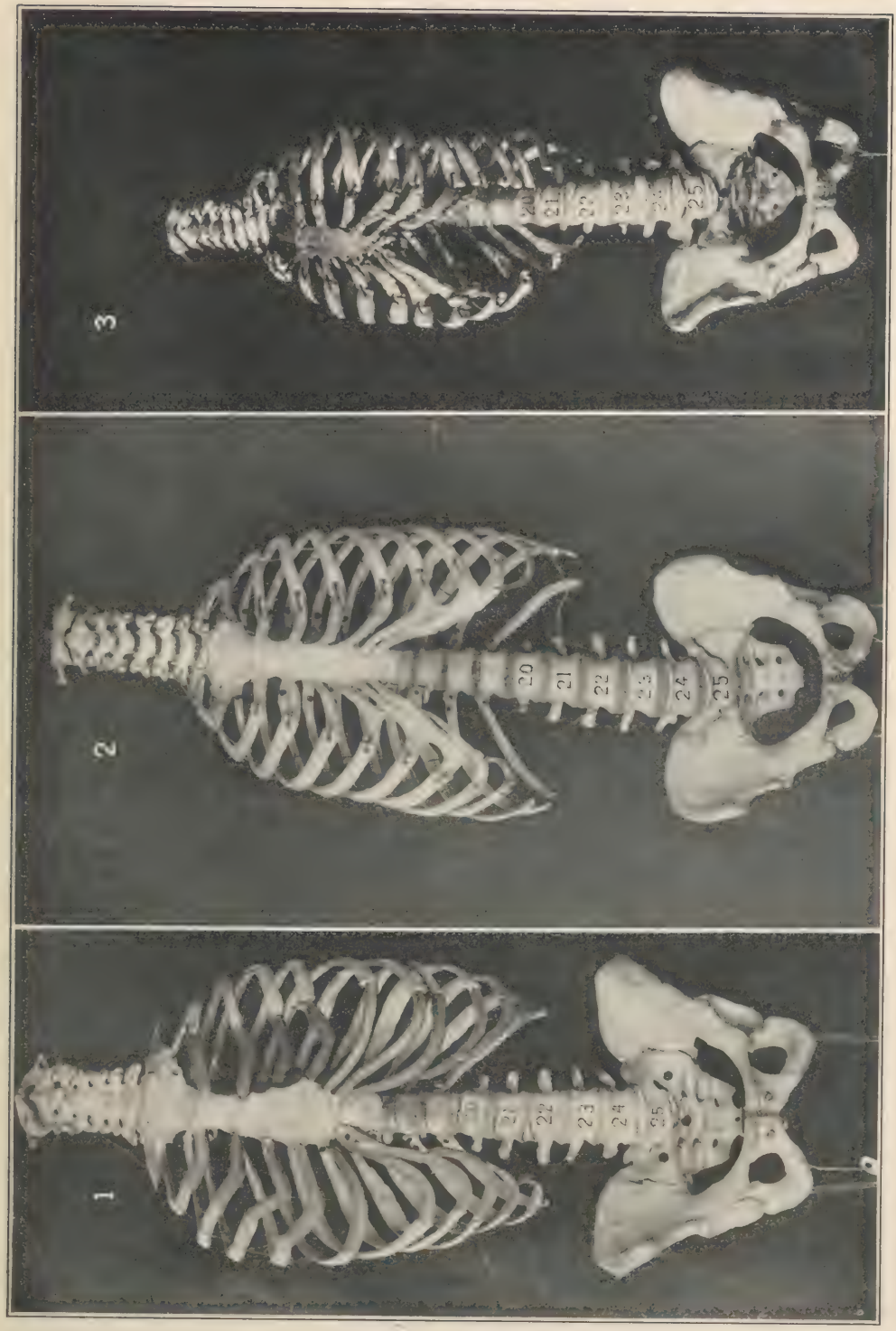
PLATE III.

Adult human instances of phyletic retardation of pelvic migration.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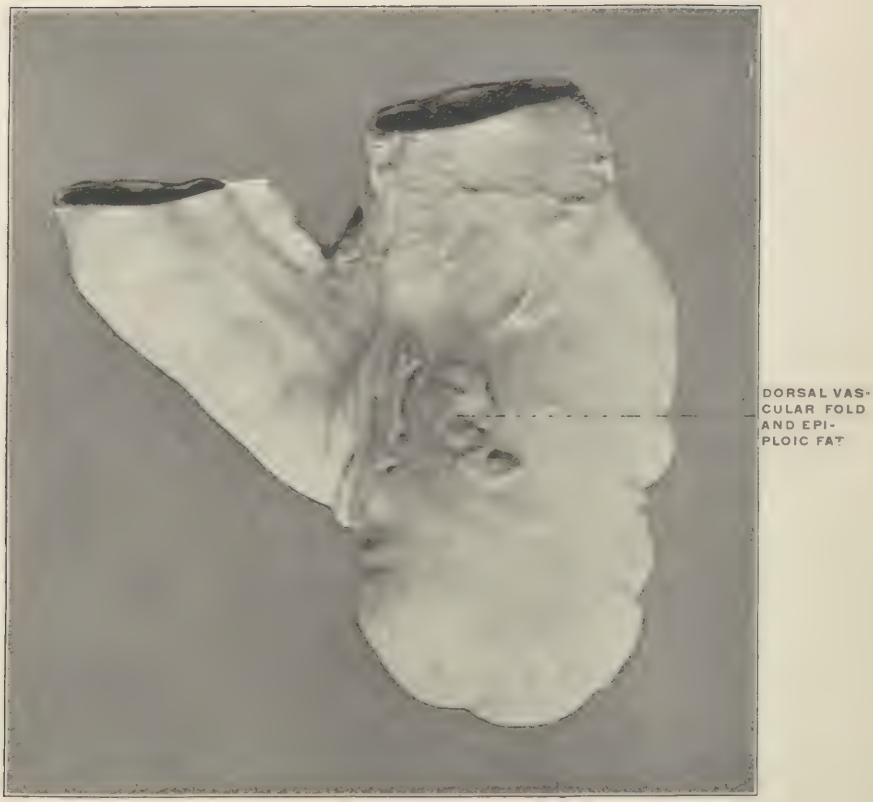

PLATE IV

Congenital absence of the Vermiform Appendix in Man.

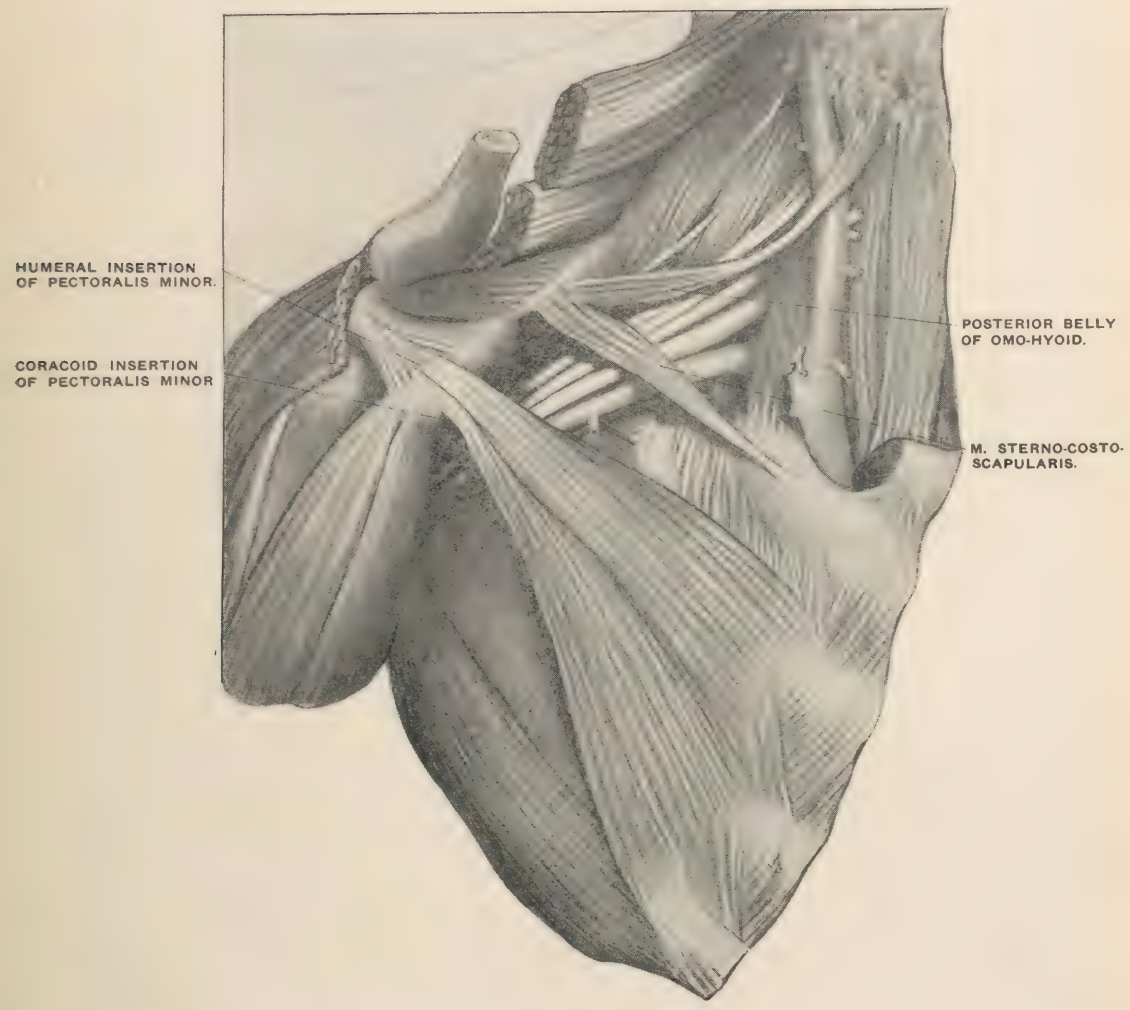

PLATE V.

Adult Human, M. Sterno-costo-scaplularis.